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This volume represents the official record of the annual meeting and is designated as the Proceedings of the 39th ASMS Conference on Mass Spectrometry and Allied Topics.

The extended abstracts contained in the Proceedings provide brief descriptions of the research presentations made at the meeting and include both oral and poster contributions. In addition, reports from workshops and interest groups and the ASMS business meeting are included.

Special thanks are given to Hank Fales for overseeing the arrangement of the technical program and Judith A. Watson and her staff of Professional Association Management for coordinating the printing and distribution of the Proceedings.

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MASS SPECTROMETRY AS AN INTEGRATED TECHNIQUE IN CHARACTERIZATION OF PROTEINS.

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Mass spectrometry has since 1985 been an integral part of the protein studies in the Protein Research Group at Odense University. At that time the first plasma desorption mass spectrometer was physically placed in the protein research laboratory, and not as other mass spectrometers, in a dedicated mass spectrometry laboratory. The mass spectrometric data were found to be highly complementary to the data obtained by conventional methods in protein chemistry, and the sensitivity was soon developed to be adequate, i.e. in the low picomole range. The very informative data combined with the robustness of the instrument, ease of operation and a high sample throughput quickly placed mass spectrometry in a central position in our protein studies. After a few years doubling of the instrument capacity was needed and recently the plasma desorption mass spectrometers have been supplemented with laser desorption and electrospray instruments.

Our mass spectrometers do not have dedicated operators. The philosophy adapted is that any researcher (student, post doc, guest, technician and even professor) can use the mass spectrometers as they would use for example UV-spectrometers and pH-meters. In the last few years, on average 20 persons have operated the instruments on a daily basis. For each instrument one person is responsible for instruction, trouble shooting and maintenance. This is only possible if the mass spectrometers are robust, reliable and equipped with extensive safety controls to eliminate operator errors, and if sample preparation is simple and well understood, reproducible and gives results at the "first shot".

The concept described above is essential for the full integration of mass spectrometry in a protein study. In general, the mass spectrum of any sample can be available within less than an hour with consumption of only low picomole quantities. Nearly all other methods to characterise a sample will be more time and sample consuming. Therefore, molecular weight determination by mass spectrometry has become the first choice to characterize any peptide or protein preparation in terms of identity and purity and to serve as a guideline for further investigations.

The protein research in our group comprises:
* primary structure determination
* characterization of post translational modifications
* structure/function studies
* protein engineering

In any of these fields the first measurement carried out once the protein is isolated is the determination of a reasonably accurate molecular weight, preferably within a few Dalton. PDMS is generally adequate for proteins up to 10 KDa, but is too inaccurate and also often fail beyond that. Based on literature and our own limited experience it seem as if LDMS and ESMS might fulfill the needs up to at least 100 KDa. ESMS has the further advantage that it seems to disclose the presence of minor components in the sample.

In protein primary structure determination a general strategy for the inclusion of mass spectrometry has been developed (1). The strategy is based on the combined use of molecular weight determination by mass spectrometry specific enzymatic cleavages, peptide separation by HPLC and sequencing by automatic Edman degradation. This scheme with slight variations is followed in all our protein sequencing.
Structure determination of protein variants, such as natural mutant proteins, isoenzymes, and artificial variants induced by protein engineering is gaining increasing actuality. For this a combined approach based on peptide mapping by HPLC and mass spectrometry is used once the sequence of the original protein is known. This "two dimensional", mapping approach has been found extremely efficient for locating the enzymatically derived subpeptides carrying the variation because chromatographically silent variants normally are disclosed by mass spectrometry (e.g. Leu → Val) and the mass spectrometrically silent variants from the chromatographic data (e.g. Lys → Gln or Glu within the error of the mass determination). Only variants silent in both dimensions need sequencing (e.g. Leu → Ile). This mapping strategy has, for example, been successfully applied to hemoglobin variants consuming as little as 2 µl of blood (2), and to isoforms of fatty acid binding proteins (3).

To our surprise the inclusion of mass spectrometry in the protein studies has shown that post translational modifications are much more frequent than we expected. On a rough estimate between half and one third of the proteins we have investigated are modified. Mass spectrometry is essential not only to reveal the presence of modified amino acid residues, but also to determine the nature of the modifying group. The structure determination of a heavily post translationally modified protein from the locust cuticle is an example of the integrated use of mass spectrometry for such studies (4).

Although the sensitivity of the mass spectrometric techniques is comparable with most of the advanced "wet" biochemical techniques, improved sensitivity is always desirable. However, a major improvement can be obtained if the integration of mass spectrometry in the biochemical process can be used to increase the overall sensitivity of a given project. This is already partially fulfilled with the present state of integration, but a further improvement might be obtained if mass spectrometry is used in combination with early analytical separation procedures, such as gel- and capillary-electrophoresis, or to characterize proteins in rather crude extracts. Both possibilities now seem to be in reach (5,6) and should place mass spectrometry as an indispensable integral element in protein studies.

References:
6 - B. Chait personal communication.
Complete carbohydrate characterization of a glycoprotein entails determining the primary structures and points of attachment of the oligosaccharide moieties. The primary structure of an oligosaccharide is defined by the following structural elements: the glycosyl residue composition; the linkage positions; the anomic configurations, absolute configurations and ring-forms of the constituent residues; the sequence of residues; and the attachment positions of any non-carbohydrate moieties (e.g., phosphate). Further complexity is introduced by the biosynthetic processing of glycoprotein oligosaccharides, which results in heterogeneous populations of structurally related oligosaccharides at individual glycosylation sites within a particular glycoprotein. In general, no single analytical method is capable of complete oligosaccharide structure elucidation; as a result, carbohydrate structure elucidation is usually carried out using several complementary methods. This lecture describes the use of mass spectrometry in conjunction with other physical (NMR), chemical (β-elimination), enzymatic (glycosidase and protease digestion) and chromatographic (high-pH anion exchange) methods to perform carbohydrate analysis of glycoproteins.

Recombinant human DNase (rDNase) is a glycoprotein with molecular weight of 30,000 and two potential sites for Asn-linked glycosylation. Approximately 10-20% of the N-linked carbohydrate of rDNase can be released by digestion with endo-β-N-acetylglucosaminidase H (endo H). When analyzed by high-pH anion exchange (HPAE) chromatography, the endo H-susceptible oligosaccharides were found to contain acidic functional groups. The acidic groups were found to be phosphate monoesters, as demonstrated by: (i) the isolation of mannose-6-phosphate after acid hydrolysis and (ii) shift in HPAE retention position to that of neutral oligosaccharides after treatment with alkaline phosphatase. After dephosphorylation, the endo H-susceptible oligosaccharides co-eluted in HPAE chromatography with two high-mannose structures (Man$_5$GlcNAc and Man$_6$GlcNAc) and one hybrid structure (NeuAcGalGlcNAcMan$_5$GlcNAc) whose structures had been determined by $^1$H NMR at 500 MHz. Liquid secondary ion mass spectrometry (LSIMS) of the p-aminobenzoic acid ethyl ester (ABEE)-derivatized oligosaccharides confirmed the underlying oligosaccharide structures and demonstrated that they were mono-phosphorylated. Collision-induced dissociation (CID)-MS was performed on the ABEE-derivatized Man$_6$GlcNAc structure to determine which mannose residue was substituted with phosphate (Fig. 1). This spectrum gave series of ions from both the reducing- and nonreducing termini that were consistent with: (i) attachment of phosphate to a linear hexose-hexose sequence (B- and C series corresponding to P-hex- and P-hex-hex-), and (ii) that the non-reducing terminal hexose residues are in a branched arrangement (X-, Y- and Z series corresponding to loss of one hexose and three hexoses, but not loss of two hexoses).

From the combined results, we conclude that the phosphate group in the Man$_6$GlcNAc structure is attached to the 6-position of the non-reducing terminal mannose residue of the Man$_6$(1-2)Man$_5$(1-3)Manβ(1-4)GlcNAc arm.
Recombinant tissue plasminogen activator (rt-PA) is a fibrin-specific plasminogen activator that is used in thrombolytic therapy in the treatment of myocardial infarction. The N-linked oligosaccharides of t-PA have been extensively characterized by several groups. Recently, a novel type of O-glycosylation was reported to occur in the EGF domain of urokinase (Kenzer et al., Biochem. Biophys. Res. Commun. 171, 401-406). We used a combination of protease- and glycosidase digestions, chemical degradation and MS to demonstrate that O-linked fucose is also present in the EGF domain of t-PA produced by several mammalian cell lines (Harris et al. Biochemistry 30, 2311-2314). The presence of O-linked fucose in t-PA was demonstrated by analysis of tryptic- and chymotryptic peptides containing Thr-61. Atmospheric pressure ionization (API) mass spectrometry was used in conjunction with carbohydrate analysis to demonstrate that fucose was covalently linked to the peptide(s). The nature of the linkage was confirmed to be O-glycosidic by the observation of destruction of Thr-61 under conditions that promote β-elimination. The linkage between fucose and threonine was found to be susceptible to α-fucosidase digestion and, therefore, has the α anomeric configuration. To examine whether O-fucosylation of t-PA was dependent on the type of mammalian cell used for expression, we examined t-PA produced in Chinese hamster ovary (CHO), Bowes melanoma and human embryonic kidney cells by reversed-phase HPLC peptide mapping and by API-MS of isolated peptides. All three cell lines were found to fucosylate Thr-61, suggesting that this is a cell-line-independent modification.
MASS SPECTROMETRY AS AN INTEGRATED TECHNIQUE IN LIPID RESEARCH

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Phospholipids are one of the principal components of cell membranes. They exist as a complex mixture of molecular species containing a variety of head groups and fatty acyl moieties. The physical and chemical properties of cell membranes are largely dependent on their phospholipid composition, and especially on their polyunsaturate content. Upon proper stimulation, polyunsaturated fatty acids are released from phospholipids and metabolized through various oxygenative pathways to form extremely potent bioactive compounds, such as prostaglandins and leukotrienes. Therefore, alterations in polyunsaturate content or metabolism can result in modification of cell functions which may in turn yield many pathological consequences. Our research is focused on the composition and metabolic functions of the polyunsaturates, with particular emphasis upon their modulation by ethanol or diet.

In order to investigate changes in phospholipid composition, a technique which can efficiently analyze phospholipid molecular species was developed using thermospray LC/MS. Molecular ion species, and mono- and diglyceride fragments, as well as head group ions which are distinctive to each phospholipid class, are produced using the filament-on thermospray technique, as is shown in Figure 1 for 18:0, 18:1-PS. Therefore, information concerning the fatty acyl composition and class identity can be readily obtained by analyzing thermospray spectra. In conjunction with on-line reversed phase HPLC, phospholipid molecular species can be separated and detected. This approach enables rapid and efficient analysis of phospholipids from complex biological mixtures. An example is shown in Figure 2 for changes in phosphatidylcholine composition in red blood cell membranes after dietary manipulation. The changes resulting from a fish oil diet, which are a loss of w-6 molecular species and an increase of w-3 species, can be readily observed from the ion chromatograms. In addition to its use to compositional studies, this technique has great potential in the investigation of phospholipid metabolic pathways such as transacylation, desaturation-elongation, decarboxylation, phospholipase reactions or molecular rearrangements, using stable isotope-labeled phospholipids.

It has been shown that ethanol modifies the production of oxygenated fatty acid metabolites by various mechanisms such as lowering levels of polyunsaturates or directly affecting various oxygenative pathways. Docosahexaenoic acid (22:6w3) is the main polyunsaturated fatty acid in brain, and it has been shown that the level of this fatty acid decreases with ethanol treatment. Since it was believed in previous reports that arachidonic acid (20:4w6) is lipoxygenated in the brain, it was therefore thought possible that 22:6w3 could also be subject to lipoxygenation in brain. This, in turn, raised the possibility that the effect of ethanol upon brain could be mediated by one or many of the oxygenated metabolites formed from 22:6w3. When 22:6w3 was incubated with the rat brain homogenate or slices, various hydroxylated 22:6w3 was observed. They were characterized using thermospray LC/MS, GC/MS and chiral phase HPLC with the aid of standards, which were prepared by reactions with soybean lipoxygenase and platelets as well as autooxidation. However, stereochemical analysis by chiral phase HPLC indicated that they were mainly racemic mixtures. Similar results were obtained for arachidonic acid. This indicated that hydroxylation occurs in the brain, not by lipoxygenation but by peroxidation. When endogenous production of various hydroxylated compounds were measured in the presence of deuterated standards, using NCI-MS after pentafluorobenzyl derivatization, hydrogenation and trimethylsilylation, subnanogram levels of peroxidation products were observed from the 1 g of brain tissue.
Figure 1. Thermospray spectrum of 2 µg of 16:0, 18:1-PS. DG=diglyceryl fragment resulting from the loss of the head group from the intact molecule MG= monoglyceryl fragment resulting from the loss of either fatty acyl group from the diglyceryl fragment.

Figure 2. Ion chromatograms reconstructed with diglyceride ions of major phosphatidylcholine molecular species of rat erythrocytes obtained after feeding a corn oil or fish oil diet for 21 days. To separate molecular species, an Ultrasphere-ODS column (4.6 mm x 7.5 cm, 3µ) and a mixture of methanol:hexane:0.1M ammonium acetate (500:25:25) was used with a flow rate of 1 mL/min.
Arginine Isotopic Ratio Measurements Using a Perfluoroalkyl-oxazolinone (FOX) Derivative

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Arginine metabolism is of interest because of its relation to the urea cycle, the immune response (nitric oxide formation) and overall nitrogen balance. A simplified, mass spectrometric analysis of stable-isotopically labeled arginine would be useful in the investigation of its in vivo metabolism.

Typically α-acyl, alkyl esters have been used for amino acid GC/MS analysis, but these undergo extensive fragmentation in an El source with losses of the guanido and acid moieties. Cyclized-guanido and TBDMS derivatives fared no better, thus requiring the use of CI sources for the analysis of various and multiply-labeled arginine analogues. Previously, this laboratory and others have reported the use of 2-perfluoroalkyl-3-oxazolin-5-one (FOX) derivatives of phenylalanine for GC/MS analysis. This derivative gave a strong molecular ion in El and was useful for both various labeled mole fraction and analyte concentration determinations. Additionally, the derivatization was a simple, one-pot procedure, minimizing necessary sample work up and avoiding loss of the more volatile amino acid derivatives.

Modification of the previously reported derivatizing reagent mixture of acetonitrile (CH₃CN) and perfluoroacyl anhydride (in this case, trifluoroacetic anhydride, TFAA) was required in order to avoid problems associated with heating amidines and guanidines in the presence of CH₃CN. 1,2-dichloroethane was substituted in a 4:1 (v:v) ratio with TFAA. Optimal reaction conditions for 250 μL of plasma were determined by comparing GC peak area ratios of the derivatized arginine with a known amount of added external standard, N-TFA, norleucine methyl ester. The final procedure required heating the isolated amino acids with 100 μL of the 4:1 reagent at 100°C for 120 minutes. No other preparatory steps were necessary.

PCI and NCI analysis of the derivative show very strong ions at m/z 445, (M+H)⁺, and 444, M⁺, indicating the compound is a dTFA, FOX structure, most likely guanido-N,N-diTFA, FOX-arginine. The largest (or near largest) fragment by EI-MS is m/z 375, (M-CF₃)⁺; this effectively allows all labeled arginine compounds to be observed by a single ionic species.

Single calibration curves for guanido-¹³C-; 5,5-d₂- and guanido-¹⁵N₂, 5,5-d₂-arginine in the range of 0 to 1% (except d₂, range = 0 to 21%) were prepared as aqueous solutions, derivatized and measured by GC/EI-MS. Linear regression results of isotopic mole fractions versus the appropriate m/z area ratios were r² = 0.994 / limit of detection (LOD) = 0.996/1.4%; and 0.997/0.6% respectively. Multiple isotope calibrants were prepared with various mixtures of the above labeled compounds and measured. Calibration of area ratios versus mole ratios was accomplished using an inverse multivariate (P matrix) method. Correlation coefficients for ¹³C- and d₂- compounds were 0.959 and 0.981 respectively; the ¹⁵N₂, d₂-arginine signal area was unaffected by the presence of either other analogue.

It was shown that arginine concentration could also be determined by this method. Homoarginine was used as an internal standard and arginine/homoarginine molar ratio standards were prepared in the range of 0.225 to 2.05. The standards were derivatized and measured in the above manner with homoarginine showing an (M-CF₃)⁺ ion at m/z 309. Linear regression of analyte/internal standard molar ratios versus m/z area ratios gave an r² of 0.991. Five 250 μL aliquots from a single plasma pool were then taken and a known amount of the internal standard added. Concentration determinations in replicates of seven gave the following results (mean±sd): 14.6±0.5, 15.1±0.7, 15.1±0.7, 14.6±0.5, and 15.4±0.3 μmol/100mL. Inte-
sample coefficient of variation (CV) is 4.0%. HPLC analysis gave an arginine concentration of 15.1 μmol/100mL.

To ten aliquots of the same plasma pool were added various amounts (in duplicate) of guanido-13C-arginine. Analysis was made in triplicate (except sample 1B, in duplicate only) and the results are given in the table below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Theoretical Mole Fraction</th>
<th>Measured Mole Fraction</th>
<th>Std</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>0.012</td>
<td>0.016</td>
<td>0.002</td>
</tr>
<tr>
<td>1B</td>
<td>0.012</td>
<td>0.014</td>
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</tr>
<tr>
<td>2A</td>
<td>0.024</td>
<td>0.024</td>
<td>0.003</td>
</tr>
<tr>
<td>2B</td>
<td>0.024</td>
<td>0.023</td>
<td>0.002</td>
</tr>
<tr>
<td>3A</td>
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<td>0.033</td>
<td>0.001</td>
</tr>
<tr>
<td>3B</td>
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<td>0.037</td>
<td>&lt;0.001</td>
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<tr>
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<td>0.064</td>
<td>0.003</td>
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</table>

Furthermore, measurements of plasma isotopic mole fractions were made at different time points from a subject undergoing infusion of equal amounts of guanido-13C- and guanido-15N2, 5,5-d2-arginine. The values were divided into fasting and fed groups, averaged and are reported with their standard deviations below. The values are statistically the same.

<table>
<thead>
<tr>
<th>Table II</th>
<th>13C-Arg</th>
<th>15N2, d2-Arg</th>
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<tr>
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<td>mole fraction</td>
<td>mole fraction</td>
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<tr>
<td>fasting:</td>
<td>0.105±0.002</td>
<td>0.106±0.004</td>
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<tr>
<td>fed:</td>
<td>0.112±0.006</td>
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The F0x derivative of arginine was investigated as a possible alternative to N-acetyl, Nε-acetyl esters. This derivative offers the advantages of one-pot formation with minimum sample work-up time and detection of the entire molecule by GC/EI-MS. Various and multiply-labeled arginine analogues were detected and accurately quantitated either as individual or multiple analytes. Additionally, with an internal standard plasma free arginine concentration could be determined.

DETERMINATION OF PHOSPHOLIPID PRECURSORS OF ARACHIDONIC ACID INVOLVED IN LEUKOTRIENE BIOSYNTHESIS USING STABLE ISOTOPE LABELLING AND FAST ATOM BOMBARDMENT TANDEM MASS SPECTROMETRY.

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National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206

For the past thirty years there has been extensive investigation into the biochemistry of the arachidonic acid (AA) cascade of metabolites. This is primarily because of the potent biological activities of many of the AA metabolites, such as prostaglandins, thromboxane, and the leukotrienes. These oxidative metabolites of AA have been implicated as mediators of many physiological and pathophysiological processes, such as inflammation and platelet aggregation. In the late 1960's it was determined that most cellular AA is esterified in the sn-2 position of glycerophospholipids (1) and also that activation of phospholipase A2, which liberates AA from phospholipids, was necessary for the production of AA metabolites (2). Therefore it is generally accepted that these bioactive substances are produced from the action of cyclooxygenase or 5-lipoxygenase on AA released from cellular phospholipids. However, no one has ever shown there to be a direct link between any specific phospholipid molecular species and AA metabolites. There are numerous molecular species of phospholipids in mammalian cells containing AA that could serve as precursors to the prostaglandins and leukotrienes, which has made it difficult to determine the identity of an exact phospholipid precursor. To investigate the source of arachidonate we are using negative ionization fast atom bombardment and tandem mass spectrometry to follow incorporation of stable isotope labelled AA into phospholipid molecular species in cultured mast cells and correlating the isotopic enrichment of the arachidonate-containing molecular species with the isotopic enrichment of leukotriene B4 (LTB4) that is produced upon stimulation of the labelled cells.

METHODS

Cell Culture Conditions- D8-AA was added to the culture medium of virally-transformed murine bone marrow-derived mast cells (1 x 10⁶ cells/ml) to achieve a fatty acid concentration of 2 μM. Anti-dinitrophenol (DNP)-IgE was added to the cells (1μg/ml). After appropriate incubation times with labelled fatty acid, the antigen stimulus, DNP-albumin, was added to activate the cells to initiate leukotriene biosynthesis. Figure 1 illustrates the protocol for a typical experiment, including the lipid isolation procedures.

Figure 1. Protocol for D8-AA incorporation into cultured mast cells, stimulation of labelled cells, and isolation of lipids. Following stimulation, the cells are centrifuged. The LTB4 is isolated from the supernatant by reverse-phase HPLC using a gradient from 55% methanol/H2O (pH 5.7) to 100% methanol. Cellular phospholipids are separated according to polar head by normal phase HPLC using a hexane:isopropanol:H2O gradient (30:40:3.3 to 30:40:7).

Mass Spectrometry- Collision induced dissociation (CID) of the [M-H]- ions from phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine, or the [M-CH3]- ion from phosphatidylcholine molecular species, yields abundant carboxylate anions that correspond to the fatty acids esterified on the glycerol backbone of the phospholipids. Isotopic enrichment of each potential precursor phospholipid molecular species was determined using selected reaction monitoring for the decomposition of the [M-H]- (or [M-CH3]- for GPC) ion to the arachidonate anion (m/z 303) for each molecular species and its D8 analog, which gives rise to m/z 311 upon CID. The ratio of the intensities of the ions at m/z 311 to m/z 303 then represents the ratio of labelled to unlabelled arachidonate-containing molecular species
that are potential leukotriene precursors. A Finnigan TSQ70B triple quadrupole mass spectrometer, equipped with a fast atom gun was used to obtain all the phospholipid mass spectra. The fast atom gun was operated at 1 mA with xenon accelerated to 6kV. Diethanolamine was used as the liquid matrix. Selected reaction monitoring was carried out with argon (0.5 mtorr) as the collision gas and a collision offset energy ($E_{i\text{a}}$) of 30 eV. The stable isotope enrichment in the leukotrienes was determined by measuring the ratio of the product $D_8$-LTB$_4$ to the product LTB$_4$ by electron capture negative ionization GC-MS of its pentafluorobenzyl ester-trimethylsilyl ether derivative. A Finnigan SSQ70 quadrupole mass spectrometer equipped with a Varian 3400 gas chromatograph (10m x 0.2mm DB-1 capillary column) was used for selected ion monitoring of the [M-PFB] ions.

RESULTS
Representative data is shown in figure 2. The isotopic enrichment of the phospholipid molecular species in 2(a), as assessed by the ratio of the relative abundance of m/z 311 to that of m/z 303 is 0.32. The enrichment of the LTB$_4$, as assessed by the peak height ratio of m/z 487 to 479 is 0.64. The greater enrichment of the LTB$_4$ indicates that this particular molecular species, which is less enriched, could not have been the sole precursor of the AA that was converted to the LTB$_4$.

CONCLUSIONS
The combination of negative ion FAB and tandem mass spectrometry using selected reaction monitoring can be used to assess the enrichment of stable isotope labelled arachidonic acid into cellular phospholipid molecular species. Using these techniques we have been able to follow the incorporation of labelled AA into the 23 major arachidonate-containing molecular species of cultured mast cells. No conclusive correlations between the isotopic enrichment the phospholipid molecular species and that of LTB$_4$ have been found at this time.

acknowledgement- This work was supported in part by a grant from the National Institutes of Health (HL34303).

REFERENCES
ELUCIDATION OF PRIMARY STRUCTURE AND MICROHETEROGENEITY OF PULMONARY SURFACTANT ASSOCIATED SP-C LIPOPROTEINS WITH CYSTEINYL-THIOESTER-PALMITATE RESIDUES

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Pulmonary Surfactant is a complex phospholipid-protein mixture secreted by alveolar type II pneumocytes. Among the three major surfactant associated proteins known thus far, the low molecular weight (4 kD) SP-C protein exerts important biological functions in normal respiration, predominantly by reducing surface tension at the air-liquid interface of bronchoalveoli. Mature SP-C, derived from a large (197 aa) protein precursor consists of a relatively hydrophilic N-terminal sequence with two adjacent Cys residues, followed by an extremely hydrophobic C-terminal transmembrane domain. SP-C sequences were previously obtained from cDNA data (1), while conventional protein-analytical methods proved difficult or unfeasible due to its extreme hydrophobicity. The complete amino acid sequences and primary structures of SP-C isolated from human, bovine, rat and canine lung were elucidated in this study, using 252Cf plasma desorption (PDMS) and fast atom bombardment mass spectrometry (FABMS) in combination with selective degradation reactions. Of particular interest was the identification of the bis-palmitoylated, cysteinylthioester structure of SP-C, which was established by selective deacylation and mass spectral analysis.

Several forms of human and bovine SP-C proteins were isolated from lung lavage by Sephadex LH60 chromatography and final HPLC purification, and subjected to PDMS and FABMS analysis. PD spectra yielded abundant molecular ions with high (sub-pmol) sensitivity as shown in Fig. 1 (left) for a bovine protein (m/z 4068). Molecular ions of natural SP-Cs occurred as \([M+Na]^+\), in contrast to \([M+H]^+\) ions for alkali salt-free recombinant SP-C, and were established by formation of \([M+NH_4]^+\) ions, or other alkali ion adducts. The exact molecular weight determinations were confirmed by FABMS analysis and revealed several microheterogeneous proteins, which were significantly larger compared to molecular weights derived from cDNA sequences. Covalent palmitoylated, Cys\(\gamma,\delta\)-thioester-linked structures were identified by direct PDMS analysis after selective cleavage with DTT/NH\(_4\)HCO\(_3\) (pH 8), yielding molecular ions deacylated by one and two palmitoyl residues (Fig.1; right). Palmitate was found as the only fatty acid incorporated. N-Terminal sequences and palmitoylation were further determined by combined Edman degradation/PDMS of stepwise truncated proteins (2). Notably, the direct identification of the hydrophobic regions (Arg\(^{11}\) to Leu\(^{30}\)) was obtained by sequence-specific carbenium fragment ions in the PD spectra (s. Fig. 1). The structure determination, complemented by amino acid analysis, is in accord with the recently reported palmitoylated structure of porcine SP-C (3). Thus, human SP-C consists predominantly of two proteins (34/35 residues) due to a frayed (Phe/Gly) N-terminus, while bovine SP-C showed heterogeneity by an additional N-terminal Leu and by Val/Leu exchanges in positions 21 and 22. A recombinant, non-palmitoylated human SP-C was analyzed and showed an identical sequence. By contrast, natural canine SP-C was identified as a mono-palmitoylated protein with a -Pro-Cys(palmitate)-Phe-Pro-
sequence. Of particular interest is the mass spectrometric identification of fatty acid-deacylation as a specific pathophysiological structure alteration in SP-C from patients with alveolar proteinosis (4).


Fig.1: PDMS analysis of bovine SP-C and primary structures of human and bovine SP-C proteins. Left, complete spectrum of bovine SP-C with sequence-specific fragment ions in the insert; right, direct PDMS analysis after stepwise depalmitoylation with DTT/NH$_4$HCO$_3$ (pH 8, 60 and 120 min).
Rapid Recovery of Biological Compounds from Electrophoretic Gel Slices for Subsequent Analysis by Matrix-assisted Laser Desorption Fourier Transform Mass Spectrometry

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Slab gel electrophoresis is a widely used method for the high-resolution separation of large biopolymers. Mass spectrometry, with its increasing applications in biotechnology, has recently emerged as a highly-selective detector for gel electrophoresis. Our work has focused on rapid and sensitive mass spectral techniques for analyzing biomolecules separated by agarose and polyacrylamide gels. Our approach has been to develop methods to extract nucleotides and oligonucleotides from the gel matrix prior to examination by matrix-assisted laser desorption FTMS. The accurate mass measurement and collision-induced dissociation capabilities of FTMS provide both structural and sequence information for normal and modified oligonucleotides.

Agarose gel electrophoresis is commonly used for the separation of nucleic acids and their restriction enzyme cleavage products. For initial studies, agarose gels were prepared in 8.2 mM tris-phosphate buffer, and standards of oligonucleotides made up in the same buffer solution were electrophoretically introduced into the gel. For recovery, a procedure originally developed by Thuring et al. [Anal. Biochem., 66, 213 (1975)] was adapted for use with mass spectrometry. In this "freeze-squeeze" procedure, the gel slice to be analyzed is excised, frozen for a few seconds in liquid nitrogen, and then manually squeezed between two pieces of Parafilm to recover the interstitial gel fluid. Experiments using radiolabelled 14C-adenosine monophosphate indicated that recovery from the gel is typically greater than 50%. The drop of extracted fluid containing the analyte is mixed with 2-pyrazinecarboxylic acid, which is used a laser desorption matrix, and transferred to the probe tip for examination by LD-FTMS. This entire extraction procedure requires less than 5 minutes. High-quality mass spectra of nucleotides and oligonucleotides can be obtained with as little as 100 picomoles of sample originally applied to the gel.

For polyacrylamide (PAGE) gels, which are typically used for large oligonucleotide and protein separations, the "freeze-squeeze" procedure is not effective due to the high density of the PAGE gels. A modified electroelution procedure has been developed to rapidly recover biomolecules from PAGE gel slices by using the fabricated device shown in Figure 1. An electrical potential of +/-30 to 60 V is applied to a platinum wire electrode to extract the biological analyte from the gel into a buffer solution. Because gel-warping and loss of spatial resolution are not important in this case, electrical currents larger than those typically employed for electrophoretic separations are used for rapid extractions ( < 5 minutes) of analytes from the PAGE gels. The buffer solution into which the analyte will be extracted from the PAGE gel does not need to be identical in composition or concentration to the buffer solution in the gel. Studies of recovery efficiencies and detection limits for the electroelution of PAGE gels indicated that picomoles of analyte could be introduced to and subsequently extracted from these gels, which is similar to results observed for the "freeze-squeeze" method for agarose gels. An additional feature of the freeze-squeeze and electroelution recovery methods is that the analyte is recovered in solution and many other types of analyses can be performed on the sample, such as electrospray MS or other spectroscopic methods.

After a biomolecule has been extracted from a gel, it can be examined and characterized with matrix-assisted laser desorption FTMS. As an example, the isomeric dimers d(5'-CT-3') and d(5'-TC-3') were electrophoretically introduced into agarose gels (500 picomoles of each dimer) and extracted with the freeze-squeeze method. The resulting extracts were mixed with the laser matrix (2-pyrazinecarboxylic acid) and examined by 266 nm laser desorption FTMS. The negative ion spectra observed for these two isomers revealed molecular weight information as well as some fragmentation. However, the collision-induced dissociation spectra of the (M-H)+ at m/z 530 for each isomer, shown in Figures 2 a-b, provided detailed structural information and revealed fragment ions which could be used to differentiate the isomers.

* Research sponsored jointly by the Office of Health and Environmental Research, U.S. Department of Energy under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc. and by the Laboratory Graduate Participation Program under contract DE-AC05-76OR00033 between the U.S. Department of Energy and Oak Ridge Associated Universities.
Electrospray ionization dramatically extends the capability for mass spectrometric analysis of proteins and oligopeptides. The facile production of multiply charged molecular cations extends the range of mass analysis, permitting the use of conventional mass analyzers of limited m/z range. Measurement of relative molecular mass for proteins of greater than 100 kilodalton have been demonstrated, and in favorable cases precision of measurement may be better than 0.005%.

Additionally, efficiency for collisional dissociation of multiply charged molecular ions is significantly enhanced permitting the production of informative singly and multiply charged product ions. We have employed these techniques for the qualitative examination of mixtures of oligopeptides and proteins from a variety of sources. These have included mixtures of proteins obtained as subfractions from the purification (e.g. gel permeation chromatography or polyacrylamide gel electrophoresis) of biological materials, and mixtures of oligopeptides derived from the partial or complete digestion with specific proteases. We have demonstrated the applicability of electrospray ionization mass spectrometry and tandem mass spectrometry in the analysis of mixtures of bovine eye lens proteins, and the products of partial trypsinolysis of mutant calmodulins.

Although we have observed a wide variation in the electrospray ionization response among proteins of different provenance in both model and applied studies, we have so far not observed substantial discrimination in electrospray ionization among similar constituents in the analysis of mixtures. We note particularly that this is the case for tryptic fragments of proteins. This absence of significant discrimination greatly facilitates analysis particularly in the evaluation of the homogeneity of protein preparations and proteolysis based "mapping" experiments. Mass measurement precision and accuracy (typically ± 0.05 % to ± 0.005 %) from the electrospray ionization mass spectrum of the constituents of protein and oligopeptide mixtures are superior to those obtainable by techniques such as denaturing gel electrophoresis. Such increased accuracy and precision can dramatically enhance specificity of the analysis. Additionally the mass spectral resolution permits the confident distinction among species unresolved by conventional techniques. In electrospray ionization, with the increase in the relative molecular mass of the majority of analytes, there is generally an increase in the extent of multiple charging, and an increase in the number peaks (i.e. different charge states) in the spectrum. In the analysis of mixtures of proteins, these increases result in significant spectral congestion, which places higher demands on m/z resolution required for successful analysis, reduction in the ability of the method to accommodate complex mixtures and may reduce the dynamic range of the analysis.

The electrospray ionization of mixtures of proteins may be interpreted by inspection in which the association among the peaks observed in the mixed spectrum are computed to identify the overlapping distributions of multiply charged ions. Alternatively, computer algorithms may often be applied which recognize the harmonic relation among the peaks in the spectrum and deconvolute the data to provide an uncomplicated view of the composition of the mixture. An example of a mixed spectrum amenable to either approach is shown in Figure 1 for the electrospray ionization mass spectrum of an HPLC subfraction of the βL fraction of bovine lens crystallin obtained by gel permeation chromatography. The manual approach permits the recognition of three principal constituents of relative molecular mass 20,080.5 ± 3.0, 23,194.7 ± 3.5 and 24,244.4 ± 1.9 daltons respectively. These values are in good agreement with those obtained by computer deconvolution shown in Figure 1(b). It is arguable that these data are potentially more accurate since they arise from a treatment of the full array of mass spectral information. The success of this latter approach depends on the presence of a sufficient number of peaks in the spectrum to represent the
harmonicity of distribution of multiply charged ions. Where the mixtures contain constituents affording few peaks in relatively low charge states (or where the mixture contains such materials together with constituents showing a number of higher charge states) the peak assignments still rely on the manual approach and incur a somewhat greater uncertainty. As the number of charge states decreases, the possibilities of misassignment increase. Where the constituents mixtures vary widely in size and thus in charge state of the distributions of molecular ions, we have found that “high” versus “low” charge state ions may be distinguished by evaluating their relative efficiencies in the production of secondary electrons in pulse counting ion detection experiments. The physical basis of this differential behavior of multiply charged ions in the production of secondary electrons is the object of present investigations. However, this observation suggests the possibility of the crude classification of ions according to their charge state which might facilitate the interpretation of mass spectra of complex mixtures.

References
S. Brokerhoff, C.G. Edmonds and T.N. Davis, manuscript in preparation.

Acknowledgment
We gratefully acknowledge Drs. J. Zhou and I. Jardine of Finnigan MAT, San Jose, CA for access to their computational tools for the deconvolution of mixed electrospray ionization mass spectra. This research is supported by Exploratory Research of the Molecular Science Research Center at the Pacific Northwest Laboratory (DE-AC06-76RLO), the National Science Foundation, Instrumentation and Instrument Development Program (DIR 8908096) and the National Institutes of Health, National Center for Human Genome Research (HG00327). Pacific Northwest Laboratory is operated by Battelle Memorial Institute.
PLASMA DESORPTION MASS SPECTROMETRY OF LIPID A FROM ENTEROBACTER AGGLOMERANS

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2. Southern Regional Research Center, USDA, New Orleans, LA 70124
3. Dept. of Biochemistry, Choppin Hall, Louisiana State University, Baton Rouge, LA 70803

Enterobacter agglomerans is a gram-negative bacterium commonly present in field cotton. This bacterium is known to produce lipopolysaccharide (LPS), a type of endotoxin. Inhalation of cotton dust containing LPS may lead to byssinosis, a pulmonary disease afflicting textile mill workers exposed to cotton fiber and dust in the workplace [1]. The toxic activity of LPS is thought to originate from a portion of the molecule called "Lipid A" [2,3] which is liberated from LPS via gentle acid hydrolysis. The exact structure of Lipid A produced by E. agglomerans has not been previously elucidated. Many of the known Lipid A's contain two glucosamine moieties joined by an ether linkage. Fatty acid ester and amide side chains are usually present on each sugar moiety, and one or more phosphate groups is typically attached. Many varieties of Lipid A may be produced by a single bacterium.

The goal of this study is to identify the molecular weights and ester-linked side chains present on the various Lipid A's produced by E. agglomerans. Plasma Desorption Mass Spectrometry (PDMS) has been performed on treated LPS preparations containing monophosphoryl Lipid A. The PDMS instrument used was a BIO-ION 20 (BIO-ION Nordic, Uppsala, Sweden). Positive and negative ion spectra of the Lipid A mixture suggest that at least four different Lipid A structures are present. The average molecular weights of the four monophosphorylated neutral molecules are 1973, 1957, 1734, and 1718 daltons. In the positive ion mode, sodium adducts of these molecules are observed, whereas in the negative ion mode, (M-H)^+ ions appear. Fragmentations are quite abundant in both positive and negative ion modes, and the observed ions correspond to the presence of ester-linked lauroyl, myristoyl, and hydroxymyristoyl side chains.

If the Lipid A is taken through a dephosphorylation step prior to PDMS analysis, desorption characteristics in the positive ion mode improve dramatically, and the signal-to-noise ratio increases significantly relative to phosphorylated Lipid A. The positive ion spectrum of dephosphorylated Lipid A is shown in Figure 1. Postulated empirical formulas for the observed peaks are given in Table 1 along with proposed side-chain losses from A4, B4, C4, and D4 (the four dephosphorylated analogs of the above listed monophosphorylated neutral molecules, respectively).

The combined information provided by positive and negative ion mass spectra reveal that E. agglomerans monophosphoryl Lipid A having a molecular weight of 1957 daltons appears to be structurally similar to a well-characterized Lipid A produced by Salmonella minnesota (also 1957 daltons) [4]. Moreover, the E. agglomerans monophosphoryl Lipid A having a molecular weight of 1718 daltons appears to be structurally similar to a well-characterized Lipid A produced by Escherichia coli (also 1718 daltons) [5]. The two Lipid A structures having molecular weights of 1973 and 1734 daltons differ from the above structures (respectively) in that each appears to contain an additional oxygen atom on a fatty acid ester side chain attached to the distal subunit.

References:
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Figure 1:
Positive Ion
Plasma Desorption
Mass Spectrum of
dephosphorylated
Lipid A from
*Enterobacter agglomerans.*
Sample size = 6 ug;
acceleration voltage
= 18 kV;
signal was acquired for
approximately 1 hour.

Table 1:

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<th>m/z</th>
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<tr>
<td>1008</td>
<td>[C60H112NO10]+</td>
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<td>Oxonium Ion</td>
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</table>

†HM=hydroxymyristoyl
P=palmityl
M=myristoyl
L=lauroyl
CHARACTERIZATION OF SOLUBLE NOD-GENE-DEPENDENT FACTORS FROM RHIZOBIUM MELiloti CULTURES. E. Morrey Atkinson1, *Kym F. Faul12, David W. Ehrhardt1, Kenneth Conklin2 and Sharon Long1. 1Department of Biology, Stanford University, and 2Department of Psychiatry and Biobehavioral Sciences and the Neuropsychiatric Institute, UCLA School of Medicine, Los Angeles, California.

The symbiotic relationship between plants of the family Leguminosae and soil-borne Rhizobium bacteria is responsible for a major proportion of planet Earth's annual conversion of atmospheric nitrogen to organic nitrogen. This crucial component of the Nitrogen Cycle takes place in multicellular root nodules which harbour a population of bacteria. Root nodule formation is a complex process in which microscopic extensions of the epidermal cells (root hairs, which are normally involved in water and nutrient uptake) are distorted and invaded by bacteria: plant and bacterial cell division then takes place resulting in the formation of a morphologically distinct nodule usually visible to the naked eye.

Wild type (effective at initiating nodulation) and mutant strains (ineffective at initiating nodulation, so-called nod gene mutants) of Rhizobium meliloti were available. We therefore chose a novel approach to isolating the compound(s) produced by the bacteria which are responsible for root hair deformation and root cell division. This approach involved comparison of the chromatographic profiles of extracts of both strains in which we sought compounds in the culture supernatant from the wild type strain which were absent in the culture supernatant from the nod gene mutant strain.

Reverse phase high pressure liquid chromatograms of butanol extracts revealed the presence of a major peak of UV absorbing material in supernatants of the wild-type Rhizobium which was absent in the extracts from supernatants of nod gene mutants (Figure 1A). Biological tests revealed that this material caused root hair deformation and root cell division when applied to alfalfa seedlings. Further chromatography in the presence of a quaternary ammonium ion-pair reagent resolved the peak of UV absorbing material into several components (Figure 1B). Final purification of the resulting fractions was achieved in the absence of ion-pair reagent (Figures 1C & D). FAB mass spectrometry of the resolved materials was highly informative but required caution in interpretation. For example, mass spectra on two fractions at a relatively early stage in the purification (estimated on the basis of UV adsorption profile at around 70% pure) suggested the presence of two non-sulfated analogues of the compound reported at about the same time by Lerouge et al* (Figures 2A & C). However, mass spectra on the final purified fractions clearly indicated the presence of a sulfate moiety on each compound from the loss of 80 mass units from the molecular ions, and in one case showed the presence of an additional saccharide unit which had not previously been evident (Figures 2B & D).

Although final structural elucidation is awaiting the results of our on-going NMR and chemical degradation studies, the mass spectral data are consistent with the presence of two major components and at least two minor components in the original peak of UV absorbing material. One of the major components is assigned as an acetylated analogue of the compound isolated and structurally characterised by Lerouge et al* (Figure 3A); the other major component is assigned as the pentameric form of this modified saccharide (Figure 3C). The location of the additional acetic moiety on the non-reducing end of these two molecules is inferred from the FAB mass spectral pattern, but exact positional assignment of this acetate group has yet to be achieved. Two of the minor components of the mixture appear as the corresponding de-acetylated forms of the major components in which the additional acetate on the non-reducing end is missing (Figures 3B & D): the mass spectra of one of these peaks is identical to that of the compound reported by Lerouge et al (Figure 3B). Activity of these compounds in root hair deformation and root cell division assays is currently being assessed.

Figure 1. HPLC purification of nod factors: A) Chromatogram of crude butanol extract from the
culture supernatant of wild-type Sinapis alba; B) Shaded area from A) rechromatographed
in the presence of cetyltrimethylammonium bromide (CTAB). C) & D) Appropriate fractions from
B) were re-chromatographed in the absence of CTAB for final purification.

Figure 2. FAB mass spectra of partially purified (A & C, estimated at about 70% purity) and final
HPLC (D & O, estimated at greater than 95% purity) fractions. Data acquisition was with a multi-
channel analyzer (between 20 and 50 spectra accumulated per sample) using thioglycerol/nitro-
benzyl alcohol matrix (1% trifluoroacetic acid).

Figure 3. Proposed structures of the major (A & C) and minor (B & D) components isolated
from the culture supernatants.
The Investigation of Oligosaccharides in an External Source Quadrupole Fourier Transform Mass Spectrometer
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An external source quadrupole Fourier transform mass spectrometry (QFTMS) instrument has recently been constructed in our laboratory and a program initiated to develop a method for the structural elucidation of oligosaccharides. Ions are produced in a liquid-secondary ion mass spectrometry source and are injected into the analyzer cell, which is in a differentially pumped chamber, using a single stage quadrupole guide. The quadrupole guide functions only as a broad band mass filter allowing sampling of most of the ions created in the cell. The analyzer cell is contained in a homogeneous region of a 3 Tesla superconducting magnet.

Although oligopeptides have been well studied with the QFTMS technique, oligosaccharides have not been explored. A separate investigation is warranted due to the importance of oligosaccharides in cancer research and the large difference between the two classes of compounds when analyzed mass spectrometrically. Oligosaccharides pose unique problems for mass spectroscopy. Sensitivity is often poor. Fragmentation is unpredictable (abundant in some cases and nearly absent in others). Derivitization is often necessary to obtain fragmentation.

Experiments have been performed on a series of underivatized oligosaccharides from monosaccharides to a heptasaccharide (maltoheptaose, m/z 1152). Samples are dissolved in water and placed on a copper probe tip. The sample is bombarded with a Cs+ beam having 10 KV of energy. Strong parent and fragment ions are observed. Figure 1 shows a representative cation (2a) and anion (2b) spectra of the FAB/FTMS of a tetrasaccharide maltotetraose. No attempts have been made to filter out the matrix ions. However, the spectra are nearly matrix free. Both spectra show cleavages only along glycosidic bonds. In addition, pseudomolecular peaks (clusters of matrix and the oligosaccharide parent), which are commonly observed in FAB/MS of underivatized oligosaccharides, are absent. The weak matrix background and the extensive fragmentation is a consequence of the relatively long time scale involved with this method. The time between ion formation and detection are at least several milliseconds long and can be as long as several minutes. During this time matrix clusters and oligosaccharide ions decompose. This is illustrated in the spectra of lactose (Figure 2) in which the ions are injected for a period of 5 msec and detected at 1 msec (2a) and 10 msec (2b) after the injection period. A significant decrease is observed for the protonated diglycerol species (G_2H^+) when the detection delay time is lengthened. When large oligosaccharides are analyzed a similar effect is observed with the parent ions. Protonated and sodiated maltotetraose, formed by adding NaCl in the oligosaccharide solution, decrease in intensity as the detection delay time is lengthened from 2 to 7 msec. Interestingly, the decrease of the protonated species occurs more rapidly than the sodiated species. We believe that both effects are due to unimolecular decomposition. FAB ionization provides sufficient energy for the metastable decay. The decomposition is, however, slow and is observed best during long detection times.

Oligosaccharides do not produce the same high sensitivity as the similarly massed oligopeptides. Nonetheless, 6 μg of underivatized compound is readily observable. High resolution is obtained in both the anion and cation mode. A resolution of 16,000 (FWHH) for the sodiated maltotetraose (m/z 689) and 85,000 for the anion of maltose (m/z 341).

1. For Schematics of the instrument see: Gard, E.; McCullough S.; Lebrilla, C.B. in this Proceedings.
Figure 1. FAB/FTMS of maltotetraose (a) cation and (b) anion.

Figure 2. FAB/FTMS of lactose with an injection time of 5 msec and two detection time delay corresponding to 1 msec (upper) and 10 msec (lower).
APPLICATION OF FAST ATOM BOMBARDMENT MASS SPECTROMETRY TO BIOCHEMICAL STUDIES OF SECOND MESSENGERS: I, CYCLIC NUCLEOTIDES

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Cyclic nucleotides play a central role in mammalian cellular regulation and are consequently a major topic of current research interest. Each of the naturally occurring cyclic nucleotides contains a ribose ring, a heterocyclic base (BH) and a cyclic phosphate moiety, linked to the ribose ring at positions 3' and 5' or 2' and 3', with the former isomers having regulatory functions and the latter being merely products of nucleic acid degradation. One cyclic nucleotide, adenosine 3', 5'- cyclic monophosphate (cyclic AMP) mediates the action of a large number of hormones and neurotransmitters, leading to its description as a second messenger; a second cyclic nucleotide, guanosine 3',5'- cyclic monophosphate (cyclic CMP) acts in the visual cycle of the mammalian eye and as a mediator of atrial natriuretic peptide.

While not ideally susceptible to EI or CI mass spectrometric analysis, cyclic nucleotides yield useful protonated molecular ions on fast atom bombardment (FAB) MS, and characteristic fragments can be generated by collisionally induced dissociation (CID) and mass-analysed ion kinetic energy spectrum scanning (MIKES).

This provides a rapid means of differentiating the two isomeric forms of cyclic nucleotides: MIKES analysis of the protonated molecular ion from 3',5'- isomers produces spectra containing major fragments at BH* +28 and BH* +42, while that from 2',3'- isomers contains a much weaker BH* +42 fragment due to the substitution of the 2' position of the ribose ring (1,2).

![Fragmentation of 3',5'- cyclic nucleotides leading to formation of diagnostic ions.](image)

By application of this procedure to partially purified extracts of animal and plant tissues, in addition to the previously demonstrated cyclic AMP and GMP, cytidine-, uridine-, inosine- and deoxythymidine 3',5'- cyclic monophosphates (cyclic CMP, -UMP, -IMP and -dTMP respectively) have been shown to be endogenous compounds (3-5). FABMS and CID/MIKES analysis of the products of putative cytidylyl cyclase activity, an enzyme reportedly capable of the synthesis of cyclic CMP, has confirmed the identity of cyclic CMP as one of the products and, in addition, has identified 4 novel side products of the reaction, cytidine 3',5'- cyclic pyrophosphate, cytidine 2'-0-phosphate-3',5'- cyclic monophosphate, cytidine 2'-0-glutamyl-3',5'- cyclic monophosphate and cytidine 2'-0-aspartyl-3',5'- cyclic monophosphate (6). In addition to studies of naturally occurring cyclic nucleotides, FABMS/CID/MIKES analysis has been used to confirm the structure of synthetic cyclic nucleotides, for example dibutyryl cyclic nucleotides which are capable of permeating cell membranes and are used in studying the metabolic effects of the administration of exogenous cyclic nucleotides (7). More recently, cyclic nucleotide derivatives synthesized for radioimmunoassay have been subject to MS analysis, and in addition to confirming the position of succinylation and the maintenance of the integrity of the cyclic phosphate and heterocyclic base moieties, monitoring of peak heights of the diagnostic ions emanating from reactants and products of the reaction has facilitated the optimization of the reaction (8).
The latter approach indicates the validity of quantitation by FABMS; this has been used to monitor the reactions catalysed by cyclic nucleotide phosphodiesterases (9,10) and cyclic nucleotide-responsive protein kinase (8). In the case of the former, the activity was determined from

\[
\frac{[\text{NMP}]}{[\text{cNMP}]} E = \frac{[R_{BH_2} + R_{BH_2+28} + R_{BH_2+42}]}{[R_{BH_2} + R_{BH_2+28} + R_{BH_2+42}]} \times \frac{[\text{NMP}]}{[\text{cNMP}]} (m/z MH^+}
\]

where \( R_n = I_n/I_n^0 \); \( I_n = \text{peak intensity at } m/z_n \text{ in the MIKE spectrum}; \)

\( \Delta I = \text{change in peak intensity at } m/z \text{ in MIKE spectrum spiked with standard and } [\text{NMP}] \) and

\( [\text{cNMP}] \) are the concentrations of substrate and product spike and the rate of reaction from

\[
\frac{[\text{NMP}]}{[\text{cNMP}]} E \times \frac{[S_0]}{[S_0] - [\text{NMP}]} \text{ at zero time}
\]

and \( t = \text{length of incubation} \)

Close correlation between kinetic parameters determined by conventional radiometric assays and by this mass spectrometric method has been obtained.

The authors gratefully acknowledge support from The Wellcome Trust, SERC and the Royal Society.

REFERENCES cited

Acyl-Coenzyme A (CoA) compounds are intermediates in the catabolism of fats and branched-chain amino acids. The ability to differentiate isomeric acyl-CoA’s and to elucidate the structure of novel species is important in the study of certain metabolic disease states which result from enzymatic blocks in these catabolic pathways. Mass spectrometry, and the combination of chromatography and mass spectrometry, have been demonstrated to be useful in the study of acyl-CoA compounds. This report summarizes a study whose objectives were to understand the mass spectrometric behavior of acyl-CoA compounds, to identify optimum analytical modes, and to develop strategies for isomer differentiation and structure elucidation.

Experimental

A significant number and variety of acyl-CoA compounds were investigated during this study. These included acyl groups of varying chain length (C2-C18), unsaturation, branching, and functional group content (hydroxy-, carboxy-, keto-). The analytical modes investigated included FAB +/-, thermospray +/-, and electrospray +. Both quadrupole and magnetic sector instruments were employed in this study. Tandem mass spectrometry (MS/MS) studies with FAB and thermospray were accomplished on a triple quadrupole instrument (VG Trio-III) using 15eV argon collisions.

Results

Examples of the FAB and thermospray behavior of octanoyl-CoA, which is representative of these compounds, are shown in Figures 1 and 2 respectively. Molecular ion species for all compounds studied were more favorably generated by FAB than by thermospray, in both positive and negative ion mode. The electrospray spectra obtained appear similar to the FAB spectra. Although the [M-H]⁻ ion can show greater relative abundance in FAB than the [M+H]⁺ ion, it was found that in general negative ion mode had no significant advantages over positive ion mode. CID MS/MS studies on [M+H]⁺ ions generated by FAB showed little utility for either isomer differentiation or structure elucidation. However, several successful analyses were accomplished by performing CID MS/MS on the [M+H]⁺-507 ion generated by FAB or thermospray (m/z 387 for octanoyl-CoA) or the acylium ion generated by thermospray. An example is presented in Figure 3 which shows MS/MS spectra derived from 3-ketooctanoyl-CoA and an unknown which was identified as 3-ketovalproyl-CoA. The relative abundance of m/z 57 located the position of the keto group in this species.
Figure 1. Positive and negative ion FAB spectra of octanoyl-CoA.

Figure 2. Positive and negative ion thermospray spectra of octanoyl-CoA.

Figure 3. CID MS/MS spectra of the [M+H]^+ - 507 (m/z 401) ion produced by FAB from 3-ketoocctanoyl-CoA and an unknown (3-ketovalproyl-CoA).
Mass spectrometric analysis of top monolayers of biological surfaces can be achieved using the technique of static secondary ion mass spectrometry (SIMS). Static SIMS employs low primary ion doses \((10^{10}-10^{12} \text{ ions/cm}^2)\) which maximize formation of molecular or structurally significant ions. Static SIMS analyses using KeV primary ion beam energies typically penetrate only \(10-50A\) into the sample. A novel instrument configuration incorporates a high performance time-of-flight (TOF) mass spectrometer with ion image capabilities. The distribution of secondary ions emitted from the sample surface can be determined to lateral resolutions approaching \(2000A\).

A sample of rabbit adrenal tissue was fixed, shock-frozen and cryomicrotomed. After freeze-drying overnight, positive ion mass spectra and ion images were acquired of both the adrenal medulla and cortex. An abundant molecular ion for epinephrine at \((M+H)^+\) 184 could be observed in spectra from the adrenal medulla and cortex. However, the ion image of the medulla region revealed regions of higher concentration of ions for epinephrine, while the ion image of the cortex showed a uniform distribution of epinephrine ions. Only the central part of the medulla would be expected to have higher concentrations of epinephrine. The bulk concentration of epinephrine in the rabbit adrenal is approximately 0.3 ppmw.

Figure 1. This figure shows the structure of spiperone and positive-ion mass spectra of spiperone deposited on a Si wafer in a solution of MeOH (top), and mixed into gelatin and deposited on a Si wafer (middle and bottom). The samples were dried prior to analysis.
In order to further evaluate applications of this technique to biological systems such as freeze-dried soft tissue, analysis of a series of standard compounds in a variety of tissue-like matrices are being performed. These analyses will determine the dependence of ion yields from various types of compounds on the general chemical composition of the matrix and the role of non-specific binding of compounds to the matrix.

Mass spectral data of spiperone, which binds dopamine receptors, epinephrine, and a neuro-active compound of the elemental composition $C_{27}H_{35}N_6O_5F_3$ in three matrices were presented. These compounds were first dissolved in MeOH or H$_2$O and then mixed with either commercial gelatin, Matrigel™ or a 20% aqueous brain homogenate. Analytical concentrations ranged from $10^{-3}$-$10^{-5}$ M wet weight. Each sample was deposited as a drop on a Si wafer and dried at room temperature prior to analysis. Matrigel™ is a solubilized basement membrane, containing type IV collagen, heparin and proteoglycans as well as other connective tissue proteins. Gelatin is a solubilized form of connective tissue collagen and contains less carbohydrate than Matrigel™. The brain extract was prepared by homogenizing human brain tissue in 5 volumes ice cold H$_2$O (5 ml/g) with a tissue homogenizer. This mixture was centrifuged at 15,000 x g for 30 min and the supernatant removed. Typical positive-ion spectra of spiperone in gelatin and aqueous brain homogenate are shown in Figures 1 and 2.

Spiperone ran similarly in all three matrices over the concentration range of analysis. Epinephrine was just detectable over the background in either gelatin or Matrigel™, but could easily be observed in the aqueous brain homogenate over the $10^{-3}$-$10^{-5}$ M range. Compound #275 produced more abundant ions at the same concentration in gelatin compared to Matrigel™. An approximately linear relationship was observed between molecular and fragment ion abundance and concentration of these analytes over the range analyzed. The analyses demonstrate that physiologically relevant levels of the three compounds can be detected in these matrices.

![Figure 2. This figure shows two positive-ion spectra of spiperone dissolved in a 20% brain homogenate, deposited on a Si wafer and dried.](image-url)
A Pulsed Electron Beam, Variable Temperature High Pressure Mass Spectrometric Determination of Proton Affinity and Entropy Differences from Proton Exchange Equilibria of Compounds Ranging in PA's from N$_2$ to H$_2$S

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The Proton Affinities [PA] of weakly basic compounds (N$_2$ to H$_2$S) have been measured on a newly constructed High Pressure Mass Spectrometer [HPMS] incorporating several novel features$^1$. The system consists of a large vacuum chamber containing the high pressure ion source coupled to a VG 70-70 mass spectrometer whose geometry is now BE (reversed geometry). The mass spectrometer has a collision cell between the electric and magnetic sectors permitting Collision Activation Spectra of mass selected ions from the high pressure ion source to be performed. In the future, laser induced decomposition of ions will also be implemented. The mass resolution capability is much larger than most HPMS apparatus and is sufficient to resolve HCO$^+$ and C$_2$H$_5^+$. This has permitted the proton exchange equilibrium between CH$_4$ and CO to be measured in one step and in many instances, suppress interferences from isobaric ions.

The proton exchange equilibria thermochemical data derived from van't Hoff Plots; Ln(K$_w$) v 1/T; of the compounds studied in the present work are presented in Table 1 and the PA data summary in Table 2. It is apparent by inspection of Table 2, that the present PA scale does not reproduce either the 298K appearance energy [AE] data or the high level ab initio 298K proton affinities over the entire range. The PA difference between CO and C$_2$H$_4$ does not reproduce the AE data or the ab initio calculations. If the PA scale is anchored relative to CO, then ∆PA between CO$_2$, CH$_4$, N$_2$O, CO and C$_2$H$_4$ is in good agreement with the AE data and ab initio calculations as appropriate. A similar conclusion is reached for C$_3$H$_8$, H$_2$O and H$_2$S, if the PA scale is anchored to C$_2$H$_4$. This discrepancy of 3.5 kcal mol$^{-1}$ between the AE data and the current HPMS results in the ∆PA of CO and C$_2$H$_4$ has been previously observed by McMahon and Kebarle$^3$ in 1985. The $\Delta G^\circ_{298}$ for the overall reaction; CH$_4$ + C$_2$H$_4$ $\rightarrow$ C$_2$H$_5^+$ + CH$_4$ from the present work; -26.4 kcal mol$^{-1}$ is in excellent agreement with the 1985 McMahon and Kebarle$^3$ result of -26.5 kcal mol$^{-1}$. One possible source of this mismatch may be heat capacity effects on AH and AS derived from van't Hoff Plots covering disparate temperature ranges. For example, $\Delta H_{298} = -10.4$ kcal mol$^{-1}$ may be estimated from heat capacity effects for the proton transfer between N$_2$ and CO$_2$ as opposed to $\Delta H_{298} = -10.8$ kcal mol$^{-1}$ derived from the high temperature data. On the other hand, the overall ∆S for HCO$^+ +$ H$_2$O $\rightarrow$ H$_3$O$^+$ + CO is -0.4 cal mol$^{-1}$ K$^{-1}$ in excellent agreement with the estimated 298K entropy difference of -0.4 cal mol$^{-1}$ K$^{-1}$ from isoelectronic/neutral species. The $\Delta H$ of -21.0 kcal mol$^{-1}$ is in serious disagreement with the AE $\Delta H$ of -24.6 kcal mol$^{-1}$ however. High level ab initio calculations support the AE proton affinity differences between H$_2$O, C$_2$H$_4$, CO and CO$_2$ which is at variance with the data presented here. In our laboratory, work is in progress to resolve this serious discrepancy between the HPMS data with AE measurements and ab initio calculations and to firmly establish absolute proton affinities in the low basicity region.

### Table 1

**Thermochemical Data Summary**

A HPMS Proton Transfer Equilibria Study

\[ \text{AH}^+ + B \rightleftharpoons \text{BH}^+ + A \]

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<th>( \Delta S )</th>
<th>( \Delta G^0 )</th>
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### Table 2

**Proton Affinity Data Summary**

Obtained from Present HPMS Experiments

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<th>Base</th>
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<th>PA Ref. to ( \text{CO} ) kcal mol(^{-1}) (Present)</th>
<th>NIST Database kcal mol(^{-1})</th>
<th>AE 298 K kcal mol(^{-1})</th>
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The amide formed by substitution of the hydroxyl group on an amino acid with an amino group is more similar in structure to an amino acid involved in a peptide bond than is the parent amino acid. In order to better understand processes relevant to the mass spectrometry of peptides, we have studied unimolecular decompositions of gas phase protonated amides with FTMS. The initial investigation examined the chemistry of the simplest amide, formamide (Lin, Hung-Yu; Ridge, D. P.; Uggerud, E. The Unimolecular Chemistry of Protonated Formamide. Fourier Transform Mass Spectrometry and \textit{Ab Initio} Quantum Chemical Calculations. Submitted.) Reaction channels open to protonated formamide include CO loss and water loss, with CO loss the lower energy process. Quantum chemical calculations indicating the O-protonated molecule as the most stable and significant barriers to the decompositions described above were consistent with the experimental results.

Studies of glycinamide and lysinamide, obtained as the hydrochlorides, required a different experimental sequence than employed for examinations of the liquid formamide. For these studies, the reference base was admitted to the analyzer of the FTMS 2000 by a pulsed valve sequence. Ions were formed by electron impact, and ion-molecule reactions to form BH\textsuperscript{+} allowed to occur. The source was then cleared of any amide ions formed from amide neutral from the heated solids probe, and all ions other than the desired BH\textsuperscript{+} ejected from the analyzer. The conductance limit potential was lowered for an appropriate time to transfer the ionic reactant from the analyzer to the source, where ion-molecule reaction products were measured for a variety of reaction times. Reference bases used in these studies included nitrogen, methane, ethylene, methanol, ammonia, and methylamine.

Evaluation of the energetics of the proton transfer processes required measurement of the proton affinities of the amides studied. The proton affinity of glycinamide (GA) obtained by bracketing was

\[
\text{PA (ethylamine)} = 217 \text{ kcal/mol} < \text{PA (GA)} < \text{PA (n-butylamine)} = 218.4 \text{ kcal/mol.}
\]

This was in good agreement with the theoretical value of 215.3 kcal/mol determined by \textit{ab initio} quantum chemical calculations, and exceeds the PA of glycine by \textless 5 kcal/mol. The proton affinity of lysinamide determined by bracketing was

\[
\text{PA (tripropylamine)} = 234 \text{ kcal/mol} \sim \text{PA (lysinamide).}
\]

Protonated glycinamide (GAH\(^+\)) was found to exhibit two decomposition channels (only ionic products shown):

\[
\text{GAH}^+ \rightarrow (\text{GAH} - \text{CO})^+ \\
\text{GAH}^+ \rightarrow (\text{GAH} - \text{CO-NH}_3)^+
\]

Loss of CO was the lower energy process. For the systems studied, these decomposition products from the initial reactions of protonated base with neutral amide reacted further. The temporal data indicated that final products were deuterated and/or protonated amide. Kinetic data were fitted to the appropriate function to obtain an estimate of branching ratios for the two decomposition products. The difference in proton affinity between the reference base and the amide under study was used as the upper bound on the internal energy in the amide in the proton transfer process. As the internal energy deposited in the reaction decreased by a factor of ~2, the branching ratio (CO and ammonia loss relative to CO loss only) decreased by a factor of 40.

Similarly, two reaction channels were observed for protonated lysinamide (LAH\(^+\)):

\[
\text{LAH}^+ \rightarrow (\text{LAH} - \text{NH}_3)^+ \\
\text{LAH}^+ \rightarrow \text{C}_5\text{H}_{10}\text{N}^+
\]

The C\(_5\)H\(_{10}\)N\(^+\) ion appears to be analogous to the product originating in part from the R-group in lysine electron impact and chemical ionization mass spectrometry. Loss of ammonia is the lower energy process. Computations similar to those described above for glycinamide indicated that as internal energy deposited varies approximately threefold, the branching ratio (C\(_5\)H\(_{10}\)N\(^+\) relative to ammonia loss) was reduced by a factor of 15. The dissociative proton transfers observed for all three amides studied to date occur at lower internal energies for the two amino acid amides than for formamide.

Questions relating to site of protonation remain. Deuterium incorporation in some fragment ions which arise from ammonia loss indicate that deuterium-hydrogen exchange occurs rapidly in some of the protonated products. The absence of water loss from GAH\(^+\) or LAH\(^+\) suggests that the amine and/or amido sites are more basic than the oxygen in the larger amides; preliminary \textit{ab initio} calculations indicate that amino protonation is 8 kcal more stable than O-protonated glycinamide.
We have successfully coupled a laser to a conventional high-pressure mass spectrometer. The new instrument is shown in Figure 1. A pulsed Nd-YAG laser (30 mJ) is used to generate the metal ions inside the high pressure source. The ions are quickly thermalized by the high pressure (7 torr) of the bath gas. A ligand of interest, present in small concentration in the bath gas, is allowed to react with the metal ions. Multichannel scaling is used to time resolve the reaction (Figure 2). The equilibrium constant is measured from the ratio of the two traces and the pressure of the ligand. The temperature is varied and a van't Hoff plot is constructed (Figure 3). The slope and the intercept give the $\Delta H^o$ and the $\Delta S^o$ respectively for the reaction studied.

Using the newly built instrument, we have studied the interaction of weak ligands on the aluminum cation. The results are presented in Table 1. Generally speaking, the aluminum affinity increases monotonically with increasing proton affinity. We have successfully calculated the CO$_2$ and N$_2$ enthalpy changes using GAUSSIAN 90. In the case of carbon dioxide, the small entropy change is the result of a large amplitude, low frequency Al$^+$-O=C=O bending mode.

Following our initial success, we studied the reaction of organic ligands on Al$^+$. The first ligand binds very strongly and so we studied the second clustering reaction. Table 2 summarizes our findings.

The entropy changes are quite large. The data and several theoretical calculations suggest that the L-Al$^+$-L bond angle is very small (<90°). This causes a large steric hindrance and makes the $\Delta S^o$ strongly negative as shown on the right. For example, diethyl ether has several rotational modes that are hindered and thus the negative entropy change is very large. Acetonitrile, on the other hand, does not have hindered rotation and exhibits a small (normal) entropy change. The second dimethylether appears to bind to the polarized methyl of the first ether rather than on the aluminum. This is why the entropy and enthalpy changes are so small.

Reference

1. Bauschlicher, C. W et al. IJMSIP, accepted for publication
Figure 1 The newly built LAHPMS source and source chamber

Figure 2 Typical time-resolved profile

Figure 3 A van't Hoff plot: second acetone clustering on Al^+
CHEMISTRY AND PHOTOCHEMISTRY OF DOUBLY CHARGED TRANSITION METAL IONS

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Reactions of monovalent transition-metal ions with organic molecules in the gas phase have been the focus of intense investigation over the past 10 years and have involved a multitude of mass spectrometric techniques. The study of doubly charged metal ions, however, has really just recently gotten underway. Tonkyn and Weisshaar in their benchmark paper reported bond insertion and hydride transfer reactions in addition to charge transfer with Ti$^{2+}$ reacting with small alkanes in a flowing afterglow mass spectrometer. Since then, Fourier transform mass spectrometer (FTMS) has been used to monitor the reactivity of a variety of dipositive early transition metal ions including Nb$^{2+}$, LaFe$^{2+}$, Zr$^{2+}$, Ta$^{2+}$, La$^{2+}$ and Y$^{2+}$. Of particular interest is the comparison of reaction mechanisms and kinetics, as well as bond energies between the doubly charged ions and their singly charged counterparts. Kinetic energy release measurements on the reaction of M$^{2+}$ (Nb,Ta,V) with benzene to form M$^+$ and C$_6$H$_{12}^+$ obtained by studying the trapping voltage dependence of product ion intensities, has provided information on energy transfer and reaction dynamics. Photodissociation studies of these doubly charged species also holds great promise as a method for distinguishing ion structure and determining M$^{2+}$ ligand bond energies. In this talk a brief survey of this work will be presented.

As an example, Figure I shows the mass spectra obtained when LaC$_2$H$_2^{2+}$ is irradiated with light from a 2.5 kW Hg-Xe arc lamp and passed through various cut-off filters. Several photodissociation products are evident and their threshold appearance energies can be measured, yielding bond energy information. In particular, the bond energies of the acetylene, ethene and the propene ligands to La$^{2+}$ and Y$^{2+}$ are compared with the theoretical calculations in Table I. One of the fascinating results from these studies is that in some cases singly charged metal ions bind as strongly or more strongly to the ligand than the doubly charged metal ion. A simple explanation for this is that, since La$^{2+}$ has only one valence electron it binds solely by electrostatic attraction, whereas La$^+$ has two valence electrons and can insert into multiple bonds.

Table I

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Experimental ( \text{kcal/mol} )</th>
<th>Theoretical ( \text{kcal/mol} )</th>
<th>Experimental ( \text{kcal/mol} )</th>
<th>Theoretical ( \text{kcal/mol} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{C}_2\text{H}_2 )</td>
<td>42 ± 5</td>
<td>39</td>
<td>38 ± 5</td>
<td>41</td>
</tr>
<tr>
<td>( \text{C}_2\text{H}_4 )</td>
<td>42 ± 5</td>
<td>40</td>
<td>45 ± 5</td>
<td>41</td>
</tr>
<tr>
<td>( \text{C}_3\text{H}_8 )</td>
<td>41 ± 6</td>
<td>43</td>
<td>52 ± 5</td>
<td>49</td>
</tr>
<tr>
<td>( \text{C}_4\text{H}_6 )</td>
<td>85 ± 5</td>
<td>–</td>
<td>65 ± 5</td>
<td>–</td>
</tr>
</tbody>
</table>

Figure 1  Photodissociation of \( \text{LaC}_2\text{H}_4^2+ \) ion with (a) No light present, and white light with cut-off filters at (b) 437nm (c) 568nm (d) 602nm (e) 703nm (f) 745nm
Ion / molecule Reactions of Ions Derived from $V_2O_5$: a FT-ICR / MS Study

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Introduction

$V_2O_5$ is a very important catalyst used in the oxidation of a variety of organic and inorganic molecules. Various mechanisms have been proposed for its catalytic activity. The activity may be related to surface properties or to intrinsic properties of the vanadium/oxygen bonding system. Gas phase ion/molecule reactions can remove the influence of the macroscopic surface and yield information about the reactivity of vanadium and the vanadium/oxygen systems.

The oxidation of olefins and benzene derivatives has been proposed to initially involve the formation of $\pi$ complexes. To test this proposal we have studied the reactions of $V^+$ and $VO^+$ with benzene and benzene derivatives.

The oxidation of oxygen containing molecules such as alcohols is proposed to initially involve hydrogen bonding of the molecules to the $V_2O_5$ surface. Hydrogen bonding of an alcohol to a surface $V-O$ might be simulated by the study of ion/molecule reactions of $VO^+$ or $V_2O_5^+$ with alcohols. Thus we have studied the reactions of $V^+$, $VO^+$, $V_2O_5^+$ and $V_3O_8^+$ with alcohols.

Experimental

Mass spectra and ion/molecule reactions were studied using a Spectrospin CMS-47 FT-ICR mass spectrometer, equipped with a 4.7 T magnet. A cylindrical ICR cell with titanium plates (radius 30 mm x 60 mm) was used in an ultra-high vacuum chamber maintained at $10^{-9}$ mbar by a Balzers-330 turbomolecular pump.

Ions to be studied were isolated by a double ejection technique after a delay to allow thermal equilibration.

Pressed samples of $V_2O_5$ were prepared on cylindrical satellite tips which were introduced into the cell using a direct insertion probe. With the sample in the cell and a base pressure of $<1 \times 10^{-8}$ mbar, reagent gases were introduced to give an uncorrected pressure of $5 \times 10^{-8}$ mbar.

Laser ablation generation of the required ions was performed using a Nd-YAG laser (1064 nm, Spectra Physics DCR-11) focused to an area of 0.01 m.m.$^2$. In the Q-switched mode the pulse width was 8 ns producing a maximum energy at the sample surface of ~1600 MWcm$^{-2}$.

Results

$V^+$ and $VO^+$ form addition complexes with benzene and substituted benzenes. Our initial analysis of the first order kinetic rate of the disappearance of the ions showed that $VO^+$ reacted faster than $V^+$, but
fitting the kinetics for the formation of the monobenzene adduct indicated similar rates for each ion. The formation of the bis-benzene adducts occurs at an appreciably slower rate for \( \text{VC}_6\text{H}_6^+ \).

\[
\begin{align*}
\text{k}_1 & \quad \text{k}_2 \\
\text{V}^+ + \text{C}_6\text{H}_6 & \rightarrow \text{VC}_6\text{H}_6^+ \rightarrow \text{V(C}_6\text{H}_6)_2^+ & \text{k}_1=0.42, \text{k}_2=0.45 \text{ s}^{-1} \\
\text{VO}^+ + \text{C}_6\text{H}_6 & \rightarrow \text{VOC}_6\text{H}_6^+ \rightarrow \text{VO(C}_6\text{H}_6)_2^+ & \text{k}_1=0.43, \text{k}_2=0.015 \text{ s}^{-1}
\end{align*}
\]

Reactions of \( \text{V}^+ \), \( \text{VO}^+ \), \( \text{V}_2\text{O}_5^- \) and \( \text{V}_3\text{O}_8^- \) were carried out with methanol and ethanol and their deuterated derivatives. \( \text{V}^+ \) initially formed \( \text{VO}^+ \) at a much faster rate with methanol than with ethanol. Most of the reactions up to 2 s involved simple addition products. At longer reaction times products such as \( \text{VO}_4\text{C}_5\text{H}_8^+ \) and \( \text{VO}_4\text{C}_7\text{H}_{15}^+ \) were observed for the reaction of \( \text{V}^+ \) with methanol. Although we have not yet determined the structures of these ions, it is evident that the increased carbon content above the 1:4:1 (C:H:O) ratio in methanol suggests elimination of \( \text{H}_2\text{O} \) and possibly carbon-carbon bond formation.

\[
\begin{align*}
\text{V}^+ + \text{MeOH} & \rightarrow \text{VO}^+ \rightarrow \text{VO(CH}_3\text{OH})^+ \rightarrow \text{VO(CH}_3\text{OH})_2^+ & \text{VO}_4\text{C}_5\text{H}_8^+ \\
\text{V(OCH}_3\text{)}_3^+ & \rightarrow \text{V(OCH}_3\text{)}_2^+ \rightarrow \text{V(OCH}_3\text{)}_3^+ & \text{VO}_4\text{C}_7\text{H}_{15}^+
\end{align*}
\]

The anions \( \text{V}_2\text{O}_5^- \) and \( \text{V}_3\text{O}_8^- \) also react with methanol and ethanol to form addition products such as \( \text{V}_2\text{O}_5(\text{EtOH})^+ \), but again at longer reaction times unusual products are produced.

\[
\begin{align*}
\text{V}_2\text{O}_5^- + \text{MeOH} & \rightarrow \text{V}_2\text{O}_5(\text{MeOH})^- \rightarrow \text{V}_2\text{O}_5(\text{MeOH})(\text{CH}_2)^- \\
\text{V}_2\text{O}_5^- + \text{EtOH} & \rightarrow \text{V}_2\text{O}_5(\text{EtOH})^+ \rightarrow \text{V}_2\text{O}_5(\text{EtOH})(\text{C}_2\text{H}_4)^-
\end{align*}
\]

**Conclusions**

Positive ions derived from \( \text{V}_2\text{O}_5 \) form \( \pi \) complexes with benzene and some of the alkyl substituted benzenes. \( \text{V}^+ \) and \( \text{VO}^+ \) appear to form monobenzene adducts but the interaction of the second benzene is faster with the less sterically hindered \( \text{VC}_6\text{H}_6^+ \). This supports catalytic mechanisms that suggest \( \pi \) bond formation to be an initial step in the reaction of unsaturated molecules with \( \text{V}_2\text{O}_5 \).

The interaction of \( \text{V}^+ \) with methanol to form \( \text{VO}^+ \) and \( \text{VOMe}^+ \) suggests that an exposed vanadium might form a V-O bond as a first step. Addition products such as \( \text{VO(EtOH)}^+ \) might be formed by hydrogen bonding or coordination of the oxygen to the vanadium. The elimination of \( \text{H}_2\text{O} \) forming unsaturated carbon species may also aid oxidation. Thus hydrogen bonding may be an important step in the reaction of alcohols with \( \text{V}_2\text{O}_5 \) but a more definite answer must await collisional activation studies of some of the observed ions.
REACTIONS OF Si\(^+\) WITH CH\(_3\)SiH\(_3\), CH\(_3\)CH\(_3\) AND CH\(_3\)CHD\(_2\)

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Eighteen years ago, in a beam study of the formation of SiCH\(_3\)^+ in collisions of Si^+ ions with CH\(_3\)SiH\(_3\), Mayer\(^1\) observed that the peak in the kinetic energy distribution of SiCH\(_3\)^+ appeared at very low energies and was independent of the kinetic energy of the reactant Si^+. Such a characteristic is often observed in H'-abstractions from silanes which proceed by a direct process that approaches spectator stripping. If CH\(_3\)SiH\(_3\) is replaced by CH\(_3\)SiD\(_3\), still only the product SiCH\(_3\)^+ is observed, thus confirming the direct nature of the reaction. By analogy to H'-abstraction these results could be explained by a direct H\(_3\) (or D\(_3\)) transfer to the attacking Si^+ ion but this does not seem to us to be very likely. Indeed, we much prefer an alternate explanation in which the reaction proceeds via an inversion process as depicted in (1), viz.

\[
\text{Si}^+ + \text{CH}_3\text{SiH}_3 \rightarrow \text{SiCH}_3^+ + \text{SiH}_3
\]  

Since the masses of the attacking particle and the leaving particle are not very different, SiCH\(_3\)^+ would, within our energy resolution, be formed with zero kinetic energy and no scrambling of H-D would occur.

We were interested in learning if a methyl derivative with chemically identical but physically different ends would exhibit the inversion phenomenon on both ends and towards that end we studied the reaction of Si^+ with CH\(_3\)CH\(_3\) and CH\(_3\)CHD\(_2\). The major product of the reaction of Si^+ with C\(_2\)H\(_6\) at a collision energy of 1.0 eV is SiCH\(_3\)^+ with smaller amounts of SiC\(_2\)H\(_4^+\), SiC\(_2\)H\(_5^+\), SiH\(_4^+\), SiH\(_2^+\) and C\(_2\)H\(_5^+\).

Replacement of CH\(_3\)CH\(_3\) by CH\(_3\)CHD\(_2\) leads to the result shown in Figure 1, namely at 1.0 eV collision energy all three possible isotopic variants, namely SiCH\(_3\)^+ (m/z=43), SiCH\(_2\)D\(_+^+\) (m/z=44) and SiCHD\(_2^+\)

![Fig. 1. Product Spectrum from Reaction of Si^+ + CH\(_3\)CHD\(_2\) at 2.0 eV (Lab.).](image)

(m/z=45) are formed. However as the collision energy is varied, as shown in Figure 2, a dramatic change occurs in the relative abundance of these products. Increasing energy decreases the intensity of SiCH\(_2\)D\(_+^+\) relative to the intensities of SiCH\(_3^+\) and SiCHD\(_2^+\) while at the same time the ratio of i(SiCHD\(_2^+\)) to i(SiCH\(_3^+\)) remains essentially constant at 0.84±0.07.
These results indicate that SiCH$_3^+$ and SiCHD$_2^+$ are formed by the same mechanism and that this mechanism is different from that responsible for the formation of SiCH$_2$D$^+$. Since formation of SiCH$_2$D$^+$ involves some H-D scrambling and is the dominant process at low collision energy, it is tempting to conclude that SiCH$_2$D$^+$ is formed via a long-lived collision while SiCH$_3^+$ and SiCHD$_2^+$ are formed by a direct process either one analogous to the inversion shown in (1) or to a stripping reaction. However, examination of the kinetic energy distributions of the three isotopic product ions shown in Figure 3, reveals that all three product ions are formed in processes that yield the product ion kinetic energy distributions characteristic of either the spectator stripping model or the modified stripping model. All three product ions are therefore formed in direct processes and not in processes involving a long lived complex. The direct formation of SiCH$_3^+$ and SiCHD$_2^+$ by direct processes is easy to visualize but the mechanism by which the H-D exchange on the methyl group occurs to form SiCH$_2$D$^+$ remains obscure.

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SEQUENTIAL REACTIONS OF SiD\textsubscript{2} WITH SiD\textsubscript{4}

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The reactions of SiD\textsubscript{2} and its product ions with SiD\textsubscript{4} have been examined experimentally and theoretically. A Fourier transform ion cyclotron resonance mass spectrometer was used to measure the reaction rates and product distributions. Critical transition state and intermediate structures were determined with high level ab initio calculations. Phase space calculations were used to make a quantitative comparison between the experimental and theoretical findings.

Ion/molecule reactions of SiD\textsubscript{2} and Si\textsubscript{2}D\textsubscript{4} with SiD\textsubscript{4} were studied at 300K with 1 - 4 x 10\textsuperscript{-7} torr of silane. The reaction of SiD\textsubscript{2} with SiD\textsubscript{4} proceeds at 1.3 ± 0.2 times the Langevin collision rate (K\textsubscript{L}) to produce SiD\textsubscript{3}, Si\textsubscript{2}D\textsubscript{3}, and Si\textsubscript{2}D\textsubscript{4} in the amounts of 0.54, 0.07, and 0.39, respectively. The reaction of Si\textsubscript{2}D\textsubscript{4} with SiD\textsubscript{4} proceeds at <0.005 K\textsubscript{L}. This is a terminal species in the sequential reactions with silane; its only reaction is slow silicon isotope exchange (0.20 ± 0.07 K\textsubscript{L}) with silane.

Theoretical calculations for the reactions of SiD\textsubscript{2} and Si\textsubscript{2}D\textsubscript{4} with SiD\textsubscript{4} were examined at the 6-31G* level with second order perturbation theory. The relative energies of the critical transition states and intermediates along the reaction path are displayed in Figures 1 and 2. The transition state energies are listed in Table I.

The calculated reaction pathway for the reaction of SiD\textsubscript{2} with SiD\textsubscript{4} is shown in Figure 1. There are two transition states, TS2 and TS3, which lead to different Si\textsubscript{2}D\textsubscript{3} isomers. The higher energy transition state, TS3, leads to the lowest energy isomer of Si\textsubscript{3}D\textsubscript{4}. Formation of SiD\textsubscript{3} and Si\textsubscript{3}D\textsubscript{3} are slightly exothermic or thermoneutral. However, there is an endothermic barrier to form Si\textsubscript{3}D\textsubscript{3}.

The calculated reaction pathway for the reaction of Si\textsubscript{2}D\textsubscript{4} with SiD\textsubscript{4} is shown in Figure 2. The two isomers of Si\textsubscript{3}D\textsubscript{3} may be distinguished experimentally by their reactivity. The higher energy form, D\textsubscript{z}SiSiD\textsuperscript{+}, reacts with SiD\textsubscript{4} to form Si\textsubscript{3}D\textsubscript{4} whereas the lower energy form, D\textsubscript{2}SiSiD\textsuperscript{+}, is unreactive with SiD\textsubscript{4}. The transition state for insertion from the D\textsubscript{2}SiSiD\textsuperscript{+} isomer, TS3, is slightly endothermic and thus inhibits rapid silicon isotope exchange.

Phase space calculations provide a means to quantitatively compare the experimental and theoretical results for the reactions of SiD\textsubscript{2} and Si\textsubscript{2}D\textsubscript{4} with SiD\textsubscript{4}. Phase space calculations use ab initio calculated vibrational and rotational frequencies for the critical structures to evaluate the dependence of transition state energies on reaction rate and product distribution. Limiting these rates and distributions to the measured values provides either unique values or upper bounds to the values for the transition state energies. The ab initio and phase space values are compared in Table I. The agreement, within 6 kcal/mol, confirms the accuracy of the experimental and theoretical values.
FIGURE 1

FIGURE 2

TABLE I

COMPARISON OF PST AND AB INITIO TRANSITION STATE ENERGIES

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Transition State</th>
<th>Energy (eV) PST</th>
<th>Energy (eV) ab Initio</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiD$_3^+$ + SiD$_4^-$ → D$_2$SiSISiD$_2^+$ + D$_4$</td>
<td>TS2</td>
<td>-0.4</td>
<td>-0.41</td>
</tr>
<tr>
<td>→ D$_2$SiSISiD$_2^+$ + D$_4$</td>
<td>TS3</td>
<td>-0.47</td>
<td>-0.61</td>
</tr>
<tr>
<td>→ SiD$_3^+$ + SiD$_4^-$ (TS4)</td>
<td></td>
<td>-0.16</td>
<td>-0.043</td>
</tr>
<tr>
<td>Si$_3$D$_2^+$ + SiD$_4^-$ → Si$_3$D$_2^+$ + D$_2$</td>
<td>TS1</td>
<td>-0.10 ± 0.02</td>
<td>+0.14</td>
</tr>
<tr>
<td></td>
<td>TS2</td>
<td>≥ -0.01</td>
<td>+0.51</td>
</tr>
</tbody>
</table>
Tetracyanoethylene (TCNE) has been shown to be a particularly effective trap for hydrocarbon free radicals and hydrogen atoms that are produced in the e-beam irradiation of a hydrocarbon buffer gas, such as methane or isobutane, in a chemical ionization ion source. The products of these radical-TCNE reactions then attach thermal electrons and appear in the electron capture mass spectrum (ECMS) of TCNE, as shown in Figure 1. While the radical-TCNE reactions that lead to these ions have previously been thought to occur in the gas phase, we provide evidence here that all of the ions in Figure 1 are produced by reactions on the walls of the ion source prior to EC.

(1) We have determined the EC rate constant of TCNE by pulsed high pressure mass spectrometry (PHPMS): it is $1.2 \times 10^{-7}$ cc s$^{-1}$. Since the rate constants of gas phase radical-neutral reactions can not exceed $1 \times 10^{-10}$ cc s$^{-1}$, it is unlikely that a gas phase radical reaction could compete effectively with EC by TCNE.

(2) In measuring the electron impact spectrum of TCNE following use of the ion source for CH$_4$/ECMS, we observe in Figure 2 an unexpected H-adduct ion. This ion is clearly due to a wall related reaction of TCNE where the ion source walls have retained H atoms after exposure to irradiated methane.

(3) When TCNE is introduced to the ion source by gas chromatography, the unexpected ions persists in time after the TCNE has passed through the ion source, as shown in Figure 3. Clearly, wall-relative processes are responsible for this.

(4) In 3 torr isobutane buffer gas, the ECMS spectrum of TCNE shown in Figure 4 is obtained. By using a pulsing sequence in which the e-beam is "on" for 100 ms and "off" for about 1 second, the temporal profile of these ions have been measured and are shown in Figure 5. Inspection of these profiles indicates that the ions of the type $(M+R-CN)^-$ are formed immediately at the start of the "on" period. This is because the neutral precursors of these ions are being formed continuously during the "off" period by reactions with radicals that are attached to the walls of the ion source. The ion of the type $(M+R)^-$ has a completely different time profile. Its intensity starts at zero and becomes non-zero only after the e-beam has been "on" for several milliseconds. This indicates that this ion is being formed by a prior reaction of TCNE with gas phase radicals.

In view of these results, it is concluded that all of the unexpected ions of the type $(M+R-CN)^-$ in Figure 1 are formed by wall-related reactions. These are thought to be formed by the following reaction sequence:

$$\text{wall} \quad \text{e} \quad \frac{\text{M}}{} \quad \rightarrow \quad (\text{M}+2\text{R}) \quad \rightarrow \quad (\text{M}+\text{R-CN})^- + \text{RCN}$$

in which R is either H, CH$_3$, or C$_2$H$_5$. The ion CH(CN)$_2^-$ is thought to be formed along with $(M+H-CN)^-$ in a branched EC reaction of the species $(R+2H)$. Second generation ions of the type $(M+2R-2CN)^-$ are formed by a repetition of the above sequence following neutralization of $(M+R-CN)^-$ on the walls of the ion source.
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Figure 1  ECMS spectrum

Figure 2  Electron Impact MS of TCNE

Figure 3  GC/MS spectrum

Figure 4  Isobutane/HEPESMS of TCNE (3 torr, 150°C)

Figure 5  TCNE in pulsed HPMS with isobutane

Tw = 100 ms  Tp = 1180 ms
Several generalizations regarding sulfur vs. oxygen reactivity have emerged from previous studies in our laboratory: sulfur and oxygen anions of comparable basicity exhibit similar nucleophilicity in $S_N2$ reactions;\(^1\) in contrast, sulfur is considerably less efficient in inducing elimination (E2) reactions;\(^2\) hydrogen/deuterium exchange in acid-base systems containing sulfur is less facile than in comparable oxygen systems.\(^3\)

To further explore the intrinsic reactivity of sulfur in the gas phase, we have carried out studies of sulfur-34 labeled anions by employing our Tandem Flowing Afterglow-Selected Ion Flow Tube.\(^4\) $^{34}\text{S}^-$, $\text{H}_3^{34}\text{S}^-$, $\text{D}_3^{34}\text{S}^-$, and $\text{CH}_3^{34}\text{S}^-$ have been generated in the flowing afterglow source from unenriched precursors and cleanly injected into the reaction flow tube for study.

The reaction of $\text{H}_{34}^-$ with hydrogen sulfide (eq. 1)

$$\text{H}_{34}^- + \text{H}_2 \rightarrow \text{H}_3^2\text{S}^- + \text{H}_2^{34}\text{S}$$

proceeds with ~50\% efficiency, indicating that complete equilibration has occurred within the ion-dipole complex. Similar results are obtained for the analogous reaction of $\text{H}_{18}^-$ with $\text{H}_2^{18}$, which undoubtedly has a larger complexation energy and longer lifetime.

The reaction of $\text{S}_{34}^-$ with hydrogen sulfide shows isotope exchange, proton transfer and hydrogen atom transfer pathways (eq. 2):

$$\text{S}_{34}^- + \text{R}_2\text{S} \rightarrow \text{S}_{32}^- + \text{R}_3\text{S}$$

The product branching ratios, however, indicate that proton abstraction dominates over H-atom abstraction and equilibrium is not achieved in the intermediate complex. In contrast, the corresponding reaction of $\text{O}_{18}^-$ with $\text{H}_2^{16}$ proceeds exclusively by isotope exchange with a reaction efficiency of 50\%.\(^8\)

The sulfur-34 anion exhibits isotope exchange with carbon disulfide (eq. 3) in only 18\% of the collisions:

$$\text{S}_{34}^- + \text{C}_2\text{S}_2 \rightarrow \text{S}_{32}^- + \text{C}_2\text{S}_{34}$$

This suggests that the $\text{CS}_3^-$ adduct is not formed with unity efficiency. In contrast, the corresponding reaction of $\text{O}_{18}^-$ with $\text{C}_2\text{O}_2$ indicates addition and complete equilibration occur on every collision.\(^8\)
The reactivity of H$^{34}$S$^-$ with carbon disulfide (eq. 4) is lower (1.1% efficiency) than that of $^{34}$S$^-$, suggesting a barrier to intramolecular proton transfer.

$$\text{H}^{34}\text{S}^- + \text{C}^{32}\text{S}_2 \rightarrow \text{H}^{32}\text{S}^- + \text{C}^{34}\text{SC}^{32}\text{S} \quad (4)$$

This is supported by a slightly reduced exchange efficiency for reaction of D$^{34}$S$^-$ with CS$_2$.

The D$^{34}$S$^-$ anion undergoes slow isotope exchange with ethylene sulfide (eq. 5):

$$\text{D}^{34}\text{S}^- + \text{CH}_2=\text{CH}_2 \rightarrow \text{D}^{32}\text{S}^- + \text{CH}_2=\text{CH}_2 \quad (5)$$

This process, which presumably occurs by ring opening, deuteron transfer and ring closure, was masked in the corresponding reaction of D$^{18}$O$^-$ with ethylene oxide where fast reactive pathways exist.$^7$

The preparation of isotopically labeled sulfur ions also allows us to probe the competition between reactive pathways. For example, a study of the reactions of $^{34}$S$^-$ and CH$_3^{34}$S$^-$ with methyl thioacetate (eq. 6,7) indicates that BAC2 rather than SN2 processes occur.

$$\text{O} \quad \text{CH}_3\text{C}^{34}\text{S}^- + \text{CH}_3\text{C}^{32}\text{S} \quad (6)$$

$$\text{CH}_3\text{C}^{34}\text{S}^- + \text{CH}_3\text{C}^{32}\text{SCH}_3 \rightarrow \text{CH}_3\text{C}^{32}\text{S}^- + \text{CH}_3\text{C}^{34}\text{SCH}_3 \quad (7)$$

In summary, our studies of sulfur-34 labeled anions indicate a lower intrinsic reactivity of sulfur relative to oxygen. Sulfur-containing systems have lower ion-dipole complexation energies, resulting in shorter lifetimes and less available energy for inducing intramolecular rearrangements. Moreover, in the sulfur systems, the proton or atom transfers must occur over larger distances. These factors give rise to the reduced exchange efficiencies and the incomplete equilibration which we have observed for the reactions of sulfur-34 labeled anions.

References
5. S. E. Barlow and V. M. Bierbaum, unpublished data.
Functional group interactions can enhance specific reactive or dissociative channels of gas-phase ions by altering ion stabilities. For example, hydroxyl groups are strong hydrogen bond acceptors, so these functional groups play important roles in mediating the ion chemistry of hydroxy-containing compounds. In this work, selective ion/molecule reactions and dissociative chemistry of a series of diols of variable functional group separation are examined and compared to the behavior of monofunctional alcohols.

The ion/molecule reactions of interest included simple proton transfer and methylene substitution, both induced by dimethyl ether ions, CH₃OCH₂⁺. A Finnigan Ion Trap Mass Spectrometer was used to study the ion/molecule reactions of the diols and alcohols and to characterize product ions via low energy collisional activation. Typically, the reagent gas pressure (dimethyl ether) was 8.0 x 10⁻⁶ torr, and the helium buffer gas was 1.0 mtorr. The ion/molecule reaction period was about 20-50 msec, and the activation time during which the supplementary ac voltage was applied was 3 msec. The ac voltage was 400 mV, with q = 0.4. All samples were introduced via a heated leak valve system. Typical sample pressure was 8 x 10⁻⁷ torr.

In the ITMS, collisional activation of the protonated diols results in losses of one or two water molecules. No other dissociation channels are observed. At higher activating voltages, the loss of two molecules of water is favored. The extent of sequential dehydration is dependent on the functional group separation. Protonated 1,3-propanediol always shows a greater relative extent of double dehydration, likely due to the unfavorable thermochemical stability of the primary carbocation formed from dehydration of the protonated 1,3-propanediol in comparison to the secondary carbocation initially formed from dehydration of the protonated 1,2-diol.

Reactions of dimethyl ether ions with the diols and alcohols yield two products of interest: (M + H)⁺ and (M* + 13)⁺, via (M⁺ (CH₃O=CH₂⁺) - CH₃OH). Several observations about functional group participation are apparent. First, simple mono-functional alcohols do not undergo methylene substitution with dimethyl ether ions under any conditions. This indicates that the presence of two functional groups is necessary for formation of (M* + 13)⁺ product ions. Second, with increasing functional group separation, the abundance of product ions decreases. In fact, in the ITMS no product ions were observed for 1,4-pentanediol, and the relative abundance of (M* + 13)⁺ ions was low for 1,4-butanediol, but very high for 1,2-butanediol and 2,3-butanediol.

For each diol, the product ion at (M* + 13)⁺ was isolated and collisionally activated. For all the diols in which the hydroxyl groups are adjacent (1,2-ethanediol, 1,2-propanediol, 2,3-butanediol, and 1,2-butanediol), the major dissociation routes are via loss of formaldehyde and loss of formaldehyde plus water. However, for the diols in which the functional groups are separated (1,4-butanediol and 1,3-propanediol), very different dissociative behavior is observed. For those diols, losses of water, methanol, and CH₂=CHOH (44 amu) are observed. The CAD spectra for the (M* + 13)⁺ adducts of 1,2-propanediol and 1,3-propanediol are shown in Figure 1.

Proposed mechanisms for formation and dissociation of the (M* + 13)⁺ adduct is shown in Figure 2 for 1,2-propanediol. One of the oxygen atoms undergoes nucleophilic attack on the methoxy methyl ether cation, followed by a hydrogen shift from the hydroxyl group to the ether oxygen and elimination of methanol. This addition process results in the methylene ether product at (M* + 13)⁺. Formaldehyde is easily lost from this adduct, as shown in the next mechanism. This may be followed by dehydration to yield an alkenyl cation (net loss of 48 amu from (M* + 13)⁺). Analogous mechanisms can be formulated for the other diols with adjacent hydroxyl groups.

For the diols in which the hydroxyl group are separated, very different mechanisms are operative. Proposed mechanisms which involve cyclization of the diol are shown in Figure 3 (for 1,3-propanediol). Again, initially one hydroxyl oxygen atom attacks the methoxy methyl ether cation with subsequent elimination of methanol. In this case, however, the resulting methylene ether may cyclize, forming a favorable six-membered ring protonated dioxane system. The dissociative routes of this (M* + 13)⁺ adduct ion include dehydration, loss of methanol, and elimination of 44 amu.

To test the validity of the mechanisms and product structures suggested, 1,3-dioxane was protonated and collisionally activated. Although the relative ion abundances are slightly different from those observed in the ITMS, the same mechanistic pathways were observed.
for the (M + 13)+ adduct of 1,3-propanediol, the mass-to-charge ratios of the fragments are the same. Models were not available for 1,4-butanediol and 1,5-pentanediol, however, the mechanisms suggested are consistent with the observed dissociation behavior—namely that dehydration may occur from a cyclic intermediate or transition state, methanol loss is observed, and loss of 44 amu is apparent.

Such functional group interactions are also important in mediating the reactive chemistry of more complex biomolecules containing multiple functional groups. For example, L-glucono-1,5-lactone, a sugar derivative that contains four hydroxyl groups and a cyclic ester group, demonstrates the formation of (M + 13)+ adducts provides structurally diagnostic information about the proximal positions of hydroxyl groups.

Functional group interactions play significant roles in the reactive and dissociative chemistry of simple diols. For example, mono-functional alcohols do not undergo methyne addition with dimethyl ether ions, and the extent of reactivity of diols is largely dependent on the close proximity of the two hydroxyl groups. Additionally, those diols with large separation of the hydroxyl groups demonstrate the capability for forming stable heteroatomic ring structures upon methyne addition. Finally, the gas-basicities of diols reflect the ability of the hydroxyl groups to favorably interact through the formation of more linear hydrogen bonds.

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LINEARIZATION OF FT/ICR/MS

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Elsewhere, we derived the spectral positions of all of the frequency-domain peaks (fundamentals, harmonics, and sidebands) associated with the motion of a trapped ion when all three fundamental modes are excited [1]. The unwanted peaks may obfuscate the interpretation of FT/ICR mass spectra, particularly ion abundance measurements. An axially excited ion is known to induce a smaller signal at the fundamental ICR orbital frequency than an ion with the same cyclotron orbital radius with little or no axial motion [1]. Furthermore, Laude [2] has given a cogent demonstration of how the combination of phased axial motion and frequency-sweep excitation results in errors in measuring relative ion abundances. By "shimming" the rf excitation field (electric field more spatially homogeneous), Wang and Marshall [3] and Hanson et al. [4] were able to reduce mass-discriminatory axial ejection during frequency-sweep excitation. In general, many problems associated with conventional ICR ion traps are traceable to the non-linearity of their excitation and detection fields.

From Reciprocity [1], if it is possible to shim or linearize the excitation electric field, it should be possible to linearize the detected signal from an ion. In fact, a pair of infinite parallel plates simultaneously linearizes the excitation electric potential field and the differential charge induced between them. The advantages of this linear behavior are: 1) the post-excitation cyclotron radii of ions with the same m/z are equal and determined solely by the rf excitation spectral magnitude at their ICR frequency (i.e., independent of the amplitudes of the magnetron and trapping oscillations); 2) excitation of the trapping oscillation is not possible and excitation of the cyclotron and magnetron motions can be performed only at the corresponding frequencies (i.e., not at harmonics or combination frequencies); 3) even for an ion with all three fundamental modes excited, only two peaks, at the cyclotron and magnetron frequencies, will be observed (no sidebands or harmonics); and 4) the magnitude of the signal at the fundamental ICR frequency of an ion is linear in ICR orbital radius and independent of the amplitudes of the trapping and magnetron oscillations.

Here, we present the design for a finite ion trap which mimics the desired linear behavior of a pair of infinite parallel plates: i.e., simultaneously linearizes the excitation and detection fields. Although reasoning based on Reciprocity suggested the proper circuit (shown in Figure 1), the proof of linearity in the excite field and received signal does not rest on this theorem. Two cross-sections through the linearized trap are shown with the electrical elements connecting the various electrodes. To achieve linearity, excitation and detection must be performed over the entire interior surface of the trap. Resistors to the side shim and end shim/trap plates provide a separate electrical route for applying/holding DC potentials. The capacitors serve to voltage-divide the excitation potential and to current-divide the received signal to produce the desired linear behavior. Experimental tests of this novel trap will be presented in a forthcoming manuscript.

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References
Figure 1. Parallel (upper) and transverse (lower) cross-sections through the linearized trap. Also shown are the necessary electrical elements for excite/detect linearity.
LASER STUDIES OF SOLVENT EFFECTS ON PHOTODISSOCIATION AND GEMINATE RECOMBINATION IN $I_2(CO_2)_n$ CLUSTER IONS

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We have employed a tandem time-of-flight (TOF) mass spectrometer in conjunction with nanosecond and picosecond laser systems to study the effects of stepwise solvation on the photodissociation and geminate recombination of an $I_2$ chromophore embedded in a cluster of CO$_2$ molecules. Cluster ions are generated by using an ion source in which a pulsed supersonic expansion of CO$_2$ seeded with $I_2$ is crossed with a continuous 1 keV electron beam. Attachment of low energy secondary electrons to $I_2$ produces $I_2$, from which larger cluster ions grow in the expansion via ion-molecule reactions. The resulting anionic clusters, $I_2(CO_2)_n$ (n≤55), are extracted from the expansion and accelerated into the tandem TOF mass spectrometer by using a pulsed electric field. A standard two-field Wiley-McLaren TOF forms the first stage of the tandem mass spectrometer and focuses the mass-selected cluster ion beam at the first spatial focus where the ions can interact with a pulsed laser. A single field reflectron forms the second stage of the spectrometer and refocuses the ion beam at an off-axis detector located at the second spatial focus.

Nanosecond laser pulses are generated by using the frequency-doubled output of a Quanta Ray GCR-3 Q-switched Nd:YAG laser to pump a Spectra-Physics PDL-1 pulsed dye laser. Irradiation of mass-selected cluster ions with nanosecond laser pulses induces photodissociation of the $I_2$ chromophore. Cross section measurements indicate that the photodestruction cross section for $I_2$ is ~2.5×10$^{-17}$ cm$^2$ at 720 nm. Furthermore, $I_2$ is the only important chromophore in these cluster ions at 720 nm, and its electronic structure is not significantly perturbed by CO$_2$ solvation. Photofragmentation of these cluster ions with the nanosecond laser produces two types of fragment ions: "caged" photofragment ions, $I_2(CO_2)_k$ (k<n), in which the surrounding CO$_2$ solvent molecules induce geminate recombination of the photodissociated $I_2$ chromophore, and "uncaged" photofragment ions, $I^-(CO_2)_m$ (m<n), in which an iodine atom has escaped from the cluster ion. Calorimetric arguments based on the number of CO$_2$ molecules evaporated from the cluster ion and the typical binding energy of a CO$_2$ molecule to the cluster ion indicate that the photodissociated $I_2$ chromophore in the "caged" photofragment ions has recombined and that the vibrational energy of the recombined $I_2$ has been largely transferred to the solvent bath. The quantum yield for recombination, or "caging fraction," is strongly dependent on cluster ion size, ranging from zero for n≤5 to unity for n≥16.

Time-resolved studies of the photodissociation/geminate recombination dynamics were performed by using picosecond laser pulses at 720 nm. These were generated in a cavity-dumped Coherent 702 dye laser synchronously pumped by a Quantronix 416 mode-locked frequency-doubled Nd:YAG laser. Amplification by using a Spectra-Physics PDA pulsed dye amplifier pumped with the frequency-doubled output of a Quanta Ray GCR-3 Q-switched Nd:YAG laser produced output pulses of ≤3 ps duration and 1 mJ pulse energy. These pulses were split to create identical pump and probe pulses for use in time-resolved pump-probe experiments. Irradiation of a mass-selected cluster ion with the pump pulse induces photodissociation of $I_2$. Following recombination and vibrational relaxation of the photodissociated $I_2$, a second photon can be absorbed; however, if the probe pulse interacts with the cluster ion prior to recombination and relaxation, the cluster ion will be transparent at 720 nm, and a transient bleach will be observed in the production of two-photon fragment ions. By monitoring this transient bleach, we have investigated the cluster ion size dependence of the timescale for recombination. These data can be modeled by using a simple sequential two-photon absorption mechanism in which the transient bleach is represented by a single exponential recovery:
In this model, $\sigma$ is the photoabsorption cross section at 720 nm, $F_n$ is the caging fraction for a given parent cluster ion at this wavelength, and $r$ is the absorption recovery time which characterizes the duration of the transient bleach and, therefore, the time required for recombination and vibrational relaxation to occur.

Our recent transient bleaching data suggest that the absorption recovery time for $I_2(CO_2)_n$ ($n=9-11$) is of the order 30 ps, while $r$ is considerably faster, of the order 15 ps, for $I_2(CO_2)_n$ ($n=13-17$). This difference in recovery time may indicate a change in the mechanism of recombination or vibrational relaxation in these cluster ions. Some insight into these recombination dynamics is provided by lowest energy structures generated in recent Monte Carlo and molecular dynamics simulations which suggest that CO$_2$ molecules begin to appear in "capping" positions along the I$_2$ internuclear axis for cluster ions of this size. These "capping" CO$_2$ molecules limit the range of motion experienced by the iodine atoms, thereby accelerating the recombination process.

Future studies will employ femtosecond laser pulses and two-color pump-probe experiments to evaluate the contributions of geminate recombination and vibrational relaxation to the observed transient bleach. In addition, other solvent systems, such as Xe, N$_2$O, CS$_2$, and OCS, and other chromophores, such as ICl$^-$ and IBr$^-$, will be investigated.

References


Currently research is underway in our laboratory to determine the primary structure of all the gene regulatory factors of the sea urchin embryo. The 100-150 proteins involved in the early stages of embryonic development will be isolated and their amino acid sequences determined by mass spectrometry and DNA sequencing techniques. To date, several proteins have been isolated by affinity chromatography and SDS-PAGE. Protein levels in these gels are typically less than 100 pmol. We have developed a general sequencing strategy that will enable us to obtain partial amino acid sequence on many of these proteins starting with 100 pmol or less sample in SDS gel slices. The following paper describes new methodology utilizing capillary HPLC coupled with electrospray ionization on a Finnigan TSQ-700 triple quadrupole mass spectrometer.

The combination of packed capillary HPLC with tandem mass spectrometry has the potential to redefine approaches for molecular analysis of proteins. As much as 50% of a protein's amino acid sequence can be characterized in the time required to perform three cycles of automated Edman degradation at sample levels below those currently required by commercial sequenators. Figure 1 illustrates the configuration used in our laboratory for coupling capillary HPLC with on-line UV detection with electrospray tandem mass spectrometry. A 0.1% trifluoroacetic acid/acetonitrile solvent system was employed for all experiments.

Typical results obtained using this capillary HPLC-MS system are displayed in Figure 2. The reconstructed ion chromatogram is from a capillary HPLC-MS analysis of a tryptic digest of a DNA polymerase (90,000 Da) isolated from thermophilic bacteria. The early eluting peaks are peptides in the mass range 500-5000 Da and the later eluting peaks are large partially digested fragments (7000-40000 Da). The large fragments are detected and the spectra are easily deconvoluted.

Protein tryptic digests were analyzed at sample levels as low as 800 fmol and shown in Figure 3 is a base peak plot from an analysis of 800 fmol of a tryptic digest of β-lactoglobulin. Collision activated dissociation (CAD) was used to obtain amino acid sequence information on peptides at the 5 pmol level. Displayed in Figure 4b is a capillary HPLC-MS/MS CAD mass spectrum recorded on (M+2H)2+ ions of a 15 residue peptide generated from a 5 pmol injection of a tryptic digest of myoglobin. Ions of type-Y" and type-B are labeled. Predicted fragment ions for the 15 residue tryptic peptide are shown in Figure 4a. Those fragment ions observed in the mass spectrum are underlined.

This technique is compatible with in situ digestion of proteins bound to membranes or in SDS-PAGE gel slices. Figure 6 is a base peak plot from a capillary HPLC-MS analysis of an in situ digestion of a DNA binding protein from embryonic sea urchin nuclear extract. Peptides were generated directly from proteolysis of protein in SDS-gel slices following the procedure of Ward et. al. with the following modification. Extractions were performed with 5% acetic acid for 12 h and were repeated 3 times. No organic solvent was used in the extraction buffer.

Using a capillary C4 HPLC column coupled with electrospray, proteins were analyzed at the low pmol level. Displayed in Figure 5a is a plot of the signal from a UV detector using the Chro program run on a DECstation 2100 (The same computer that is used to acquire data on the TSO-700) for the analysis of a mixture of 3 proteins; 1) 3.5 pmol of γ-bungarotoxin, 2) 3.5 pmol of uteroferrin, 3) 1.5 pmol of horse heart cytochrome C, using a homemade capillary C4 column (320 μm x 15 cm). The RIC from this analysis is shown in Figure 5b and the strip chart plot of the UV signal is in Figure 5c.


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Ultrasensitive Analysis of Thorium by Resonance Ionization Mass Spectrometry (RIMS)

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This communication presents results on a RIMS analysis of thorium. Thorium is of interest for geochronological and geochemical purposes. The measurement of uranium series disequilibrium is a well established and valuable approach for geochronological studies: disequilibrium between $^{234/238}\text{U}$ and $^{230}\text{Th}$ can be used to date samples younger than 350,000 years. Both continuous wave (cw) and pulsed lasers were utilized in this study for resonantly exciting and subsequently ionizing thorium. In the case of the pulsed laser RIMS experiments, two excimer laser-pumped dye lasers were used in conjunction with a 0.4 m time-of-flight mass spectrometer. The cw RIMS experiments used an Ar$^+$ laser-pumped dye laser and a second Ar$^+$ laser in combination with a single magnetic sector mass spectrometer (NBS 12-90 design).

Experiments performed with the pulsed RIMS apparatus were aimed at determining the autoionization state structure and re-determining the ionization potential (IP). By tuning one dye laser to a resonance and scanning the second laser such that the total energy was equal to or above the IP, over 150 autoionization states were determined. The IP was re-determined to be $6.311 \pm 0.002$ eV ($50000 \pm 20$ cm$^{-1}$). In addition, the typical cross section for ionization of an autoionizing state was determined to be $1 \times 10^{-15}$ cm$^2$.

Figure 1 is a RIMS spectrum of $^{232}\text{Th}$ obtained by scanning the wavelength of a cw dye laser such that two photons of the dye laser have sufficient energy for ionization of thorium. The peaks in the spectrum correspond to excited states of thorium. This spectrum demonstrates both the sensitivity and selectivity of cw ionization for RIMS. The sensitivity is evidenced by the intensities of the peaks in the scan ranging over four orders of magnitude. The selectivity of RIMS is evidenced by the ability to use any of the transitions observed in fig. 1 for resonance enhancement. Because each element has a unique set of excited states this ability discriminates against isobaric interferences. Several of the more intense transitions were selected for the experiments described below.
Further experiments included having a cw dye laser in resonance with one of several strong transitions and using the mid-UV lines (333-364 nm) of an Ar$^+$ laser for the ionizing step in the ionization process. This method of ionization provides the elemental selectivity desired with the dye laser in combination with the increased power for ionization of the Ar$^+$ laser. The Ar$^+$ laser provides approximately 80 times the ionizing power of the dye laser (4 W vs. 50 mW) and hence, should translate directly into increased ion yield. However, the ion signal did not in all cases increase by a simple factor proportional to the ratio of the laser powers.

Figure 2 shows three plots of enhancement versus Ar$^+$ laser power. Enhancement is defined as: $\text{Enh.} = \frac{\text{signal w/(Ar}^+\text{ and dye)}}{\text{signal w/dye only}}$. The linear increase in RIMS signal with increasing Ar$^+$ power demonstrates the lack of saturation and hence, the potential of increasing the ion yield by simply increasing the ionizing laser power. The differing enhancements for a given Ar$^+$ power level for each of the three transitions are a direct reflection of the autoionization state structure for thorium. If the sum photon energy of dye and argon lasers is in resonance with an autoionizing state, as we believe is the case here for some of the transitions utilized, its increased ionization cross section will contribute to an enhanced ion yield to the extent that the resonance occurs. Clearly, the knowledge of the autoionizing state structure is important for performing a RIMS experiment in order to maximize ionization efficiency and minimize sample size.

Because of thorium's relative scarcity in geologic samples, there is considerable interest in increasing ionization efficiency and decreasing sample size. Through the use of the Ar$^+$ laser in combination with a relatively low power cw dye laser, samples as small as 25 ng of $^{232}\text{Th}$ have been analysed and with ionization efficiencies approaching 1%. We believe higher ionization efficiencies will be obtained in the near future which will in turn enable the use of smaller samples.

References
Tandem Rapid Scanning Capillary GC UV-Vis/Mass Spectrometry

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A rapid scanning UV-Vis detector linked with fiber optics to a heated, remote flow cell has been coupled in series with a single stage quadrupole mass spectrometer. The chromatographic effluent from the exit line of the UV-Vis flow cell was routed into the source of a Finnigan 4023 GC-MS system, making use of a convenient hole in the transfer line oven which had originally been intended for use with a jet separator. The UV-Vis detector was a modified version of a commercially available instrument originally designed for use with HPLC. Vapor phase UV spectra and full scan mass spectra were acquired for a number of test samples, including Aroclors 1221 and 1254, gasoline, diesel oil, methyl anisoles, nitrotoluenes, and closely related PAH isomers of environmental and toxicological interest. The primary advantage of the technique is that it allows the unambiguous identification and differentiation of closely related aromatic compounds which often cannot be identified by the use of mass spectrometry alone. Isomeric PCB's, methyl anisoles, methyl naphthalenes and benzopyrenes can all be readily distinguished from one another using "on the fly" gas phase UV spectra. This has practical application wherever one has to deal with complex mixtures of aromatic hydrocarbons which can be separated on a GC column. The combined system has a linear dynamic range of about $10^4$, with UV detection limits of < 1.0 ng (s/n=3/1) when operating in the full scan mode (190-360 nm, 2 nm increment, 1.0 sec/scan) using unsubstituted biphenyl as a test solute.

Above: UV spectra normalized to the same intensity. All peaks with adequate s/n in this 1.0 ul sample yield unique UV spectra, although mass spectra of many isomeric mono and disubstituted PCBs show subtle differences at best.
Above: simultaneous mass and UV spectra were acquired for a 1/400 hexane dilution of Aroclor 1221. Both instruments were operated in the full scanning mode. GC program: 30h1, 120h3, 120-280 at 3°C/min, transfer line and UV flow cell 280°C, 1.0 meter X 0.32 mm retention gap with 30 meter X 0.32 mm DBS column. The exit line from the UV flow cell consisted of a 0.53 mm i.d. X 40 cm length of deactivated fused silica tubing, which was routed directly into the mass spec ion source.
Matrix assisted laser desorption time-of-flight mass spectrometry (MALD-TOF/MS) is a technique capable of molecular weight determination of high mass biomolecules. The high mass capability of the TOF experiment lead us to investigate the compatibility of MALD-TOF/MS with flowing sample introduction systems. We have made some progress with liquid sample introduction in the continuous flow mode using 3-nitrobenzyl alcohol. In order to better understand the requirements for optimizing this experiment, our attention shifted toward understanding the mechanism for MALD. Figure 1 presents a pictorial view of our proposed mechanism. The steps include: 1) photon absorption to produce an ensemble of $S$; 2) internal conversion of some $S$ species results in heating of a localized region; 3) the rapid heating causes ejection of "cluster species" from the surface, e.g., $[(S_0)_n-S]$ or $[(S_0)_n(A_n)_m-S]$ where $A$ represents analyte molecules; 4) the enhanced acidity of $S$ (relative to $S_0$) results in proton transfer to yield $[(S_0)_n(A_0)_m-AH^+-S']$; 5) evaporation of the cluster yields solvated adducts, $[(S_0)_n-AH']$ and $[(S_0)_n-S']$ or intact analyte molecular ions $[A+H]^+$. The proton transfer will be quenched if $S_0$ decays by radiative or non-radiative pathways, e.g., $S_0---A$. Coupling of electronic energy transfer can be eliminated by using matrices where the adiabatic transition energy ($E_t$) for the matrix molecules is less than that for the analyte (Figure 2). In general, pumping of high energy transitions, $S_0---S_0$ will be less effective for MALD because of electronic energy transfer and the coupling of these states to the triplet manifold. For example, the types of molecules that work best for

\footnote{Cheshnovsky, O.; Leutwyler, S. Chem. Phys. Lett. 1985, 121, 1.}
MALD, polar aromatics, the higher lying electronic states are more strongly coupled to the triplet manifold,\(^2\) this will be advantageous only if the acidity of the \(T,\) state is sufficient to promote the proton transfer reaction. Note: There are some cases where the acidity of the triplet is greater than the acidity of the singlet state.

\[ O = \cdot HUTIIIX \]

\[ IBUUTICN / DESOLVATION \]

\[ O \]

\[ TUSKR \]

\[ TUB \]

Collisionally activated dissociation (CAD) mass spectra have been widely used for structural characterization of organic isomeric ions. The optimum approach for using CAD to determine the relative proportions of gaseous isomeric ions in mixtures has been studied by several groups.

McLafferty et al. proposed methods for determining the isomeric composition of an ionic mixture based on the superposition principle and Beer's law. Bowers et al. concluded from their study that "the present method of quantitative structural analysis based on the superposition principle cannot generally be expected to yield reliable results". The assumption made by Bower et al. dictated that the collision cross-section be the same for both isomers. McLafferty et al. pointed out that the general validity of this assumption had not been demonstrated experimentally. Recently, Kim et al. suggested that multiple collision (low transmittance) conditions are unreliable, as scattering cross sections may be different between isomers, and proposed a quantitative method using CAD cross sections at zero pressure limit. In contrast, a study presented here indicated that multiple collision conditions introduced much less variety of CAD cross sections than that at single collision conditions for isomers with unequal scattering cross sections. However, comparative evaluation of these approaches has been hampered by the difficulty of preparing standard mixtures and by the lack of other analytical methods for gaseous isomeric ions. For the latter, we have recently shown that neutralization-reionization (NR) mass spectra forming both positive ion (NR) spectra and negative ions (NR spectra) can provide independent quantitative determinations. The present study describes the application of these complementary techniques to the quantitation of the C₂H₄O⁺ and C₄H₆⁺ ions, and evaluates the accuracy of these methods using comparison of the independent CAD, NR and NR results. The isomeric compositions reported here are calculated from the corresponding CAD or NR spectral data and relative cross sections of the reference ions measured with relatively high precision using our new personal computer system.

The C₂H₄O⁺ ions generated from butane-1,3-diol were reported by CAD studies to consist of structures of CH₃CH=O⁺ (e⁻) and CH₂=CHO⁻ (f⁻), and the proportions of these two isomers [e⁻]:[f⁻] were 1:1 measured by McLafferty et al. and 64%:34% by Kim et al. Recently, the structures of seven possible C₂H₄O⁺ isomers and their corresponding ions have been established by the neutralization-reionization techniques, which allows to evaluate these analytical methods more definitively using CAD, NR and NR spectra. In consistent with previous studies, only e⁻ and f⁻ ions have been observed from unimolecular dissociation of ionized butane-1,3-diol by both NR and NR methods. The proportions of isomer e⁻ by CAD, NR and NR measurements, are 50%, 44% and 45%, respectively, in good agreement with McLafferty's results. Obviously, Kim's results were outside the experimental error.

The mixtures of the isomeric cations vinylacetylene (a⁺), butatriene (b⁺), methylene cyclopropane (c⁺), and cyclobutadiene (d⁺) from 13 different precursors have been analyzed by both NR and NR techniques. For ionized benzene, 1,5- and 2,4-hexadiyne, 1,3- and 1,4-cyclohexadiene, 1,3,5-hexatriene, 2,4,6-cycloheptatrienone and cyclooctatetraene that apparently produce the same [a⁺]/[c⁺] ratio; the average of the NR values for c⁺ is 69.3% ± 2.1% and that of NR values is 65.5% ± 5.2%. For mixtures of four C₄H₄⁺ isomers formed from 13 different precursors, the deviation from the average for the individual NR and "NR" measurements of the most abundant...
for C₄H₄⁺⁺ isomers produced from 13 different precursors indicate the high reliability for this determination utilized CAD and NR methods based on the superposition principle and Beer's law.

REFERENCES


Figure 1. *NR* spectra (Hg 90%T/ He 30%T) (A,B,C) and *NR−* spectra (C₆H₆ 30%T) (D,E,F,) of (A,D) CH₃CH=O⁺⁺, (B,E) CH₂=CH-OH−, (C,F) C₂H₄O⁺⁺ from ionized butane-1,3-diol.
Introduction

Interest in radio frequency (rf) powered glow discharges continues to grow as is evidenced by the steadily increasing numbers of presentations at national meetings. This is not surprising given the benefits of such a discharge. RF discharges allow the direct analysis of nonconducting, as well as conducting, materials due to the d.c. bias which develops at the sample surface. Thus no dilution is required and possibilities of contamination of the sample are reduced because mixing with a conducting host matrix is not necessary. Isobaric interferences remain a limiting feature in glow discharge mass spectrometry. One possibility in dealing with such interferences in mass spectrometry is to increase the available resolution. In this work we detail major improvements in the interfacing of an rf glow discharge source to a double focusing mass spectrometer, which is able to resolve many atomic and molecular interferences.

Experimental

We have coupled an rf glow discharge to a double sector mass spectrometer (VG-9000). In order for such an interface to be successful, three electrical systems must be accommodated: 1) the rf system, 2) the high voltage accelerating potential, and 3) the grounded shielding system. The electrical, source, and probe design developed allows the efficient coupling of the rf potential to the sample, and thus supports a stable rf discharge. Isolation electronics maintain the division of the rf and high voltage systems while the high voltage necessary for extraction of the ions is efficiently coupled to the sample and the discharge cell. Equally important is the complete shielding of all rf carrying components and cables. Previous attempts at such an interface, utilizing an isolation transformer to reference all components to the high voltage accelerating potential, have led to inadequate shielding of the rf noise. With the new design signal-to-noise levels have been greatly increased ($\approx 10^4$).

The source, shown below, couples to the VG-9000 directly without mass spectrometer modifications. Samples (<3/16") and rf power are introduced via a direct insertion probe (21" x 1/2"). The stainless steel portion of the cell serves as the anode and is screw mounted to the boron nitride portion. The boron nitride allows cryo-cooling, high voltage insulation, and maintains an o-ring vacuum seal around the probe assembly. The discharge cell geometry efficiently restricts the discharge to the sample surface.
Results

The electrical system discussed above is capable of sustaining a stable discharge for both conducting and nonconducting species. Bias voltages as high as 600V have been observed and increase with increasing rf powers (15-45 watts typically). These bias potentials support the efficient atomization of solids for subsequent ionization within the discharge.

The analytical capabilities of this technique are demonstrated in the mass spectrum of strontium (3.85%) in NIST SRM 1412 multicomponent glass (shown below).

![Mass spectrum of strontium](image)

Of note is that noise limits have been greatly reduced over previous attempts of coupling the rf source to the VG-9000. The source and electrical design have resulted in 75 ppm limits of detection for strontium for a single scan. Analyte ion currents as high as $10^{-11}$ have been observed in conducting materials with single ppb detection limits having been obtained. These LOD’s (2σ) are obtained based on mass spectra acquired at a resolution of approximately 1200 with single scans and a 200 mS integration time. The observed differences in LOD’s for conducting and nonconducting species are not due to fundamental limitations in the analyses of nonconducting materials, but instead, current limitations in the source design. The source design to date has limited optimization of the plasma parameters and thus LOD’s comparable to the d.c. source are expected after minor changes in the source design are complete. Analytical characteristics of both nonconducting and conducting sample types are expected to be similar.
Trace Analysis of Atoms and Molecules on Surfaces Using Ion Beams

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Mass spectrometric detection of species desorbed from ion–bombarded surfaces has become an important methodology for trace analysis studies. Analysis of the desorbed ions forms the basis of fast atom bombardment mass spectrometry (FABMS) or secondary ion mass spectrometry (SIMS). Our efforts have been focused on utilizing multiphoton resonance ionization (MPRI) spectroscopy to ionize the neutral flux of particles (1). This approach offers several unique advantages when compared to other related methods. First, ion–beam desorption, as opposed to laser or thermal desorption, is extremely efficient at selectively removing molecules from only the top layer of a solid (2). This characteristic means that the signals are specific to the surface concentration of the analyte. As an additional bonus, the theory of ion/solid interactions is highly developed (3). This important fundamental aspect allows valuable mechanistic insight into the nature of the desorption event. Second, MPRI detection offers selective ionization by use of relatively low–power pulsed lasers (4). The low–power requirement allows the use of a large ionization volume. This aspect of the technique results in more efficient sampling than with ionization schemes based on electron impact or focused lasers. Third, the pulsed nature of the radiation adds another dimension of selectivity provided by the built–In availability of a time–of–flight (TOF) mass spectrometer detector. These detectors exhibit inherently very high transmission efficiency. Finally, MPRI can be an extremely efficient means of producing ions.

For atomic species, Hurst has shown that ionization efficiencies approaching 100% are possible for those atoms in resonance with and spatially overlapping the photon field (5). In our experiments, we have elucidated a number of factors which need to be considered in order to maximize the value of MPRI ionization. These include ion pulse width, laser beam cross section, ionization scheme and minimization of SIMS ions as a source of background (4). With proper care, we will show that it is possible to determine surface concentrations of impurities with sub–zeptomole detection limits. At present, these measurements are only feasible for well–defined targets such as high purity silicon. But a number of factors aimed at allowing the study of more complex matrices will be considered.

Special problems arise when attempting to utilize this technology for the detection of molecules on surfaces using ion beam induced desorption. Computer simulations as well as a limited number of experimental studies suggest that the molecules desorb in a variety of highly excited vibrational and rotational states, with the largest population remaining in the ground–state electronic manifold. Two approaches have been successfully pursued in connection with other desorption schemes that will likely have relevance to the ion–beam–induced desorption technique. In one scheme, the molecules are first entrained and cooled by a supersonic jet of a noble gas (6). The advantage of this step is that the target molecules are cooled into a single spectroscopic state. The MPRI process can then be highly selective and efficient. A disadvantage is that only a very small fraction of the molecules can be trapped into the jet, dramatically reducing the sensitivity. In a second approach, the molecules are ionized directly by a laser tuned to a wavelength associated with an electronic transition of the molecule. Since there are a high density of occupied vibrational and rotational states in the ground–state manifold and since each of these states can connect with a real level in the excited–state manifold, a degree of selectivity is lost. This simplified scheme, however, offers major advantages in sensitivity due to the increased sampling efficiency. There have already been elegant experimental demonstrations of attomole detection limits when this mode of operation is coupled to laser desorption (7).
Here we demonstrate that it is possible to achieve attomole detection limits for desorbed neutral molecules even within the framework of the above limitations. For rigid molecules such as polycyclic aromatic hydrocarbons (PAH), it is possible to detect primarily molecular ions by postionization with two photons of wavelength between 266 and 280 nm. The signal intensity linearly follows the surface concentration of the PAH to detection limits of ~1 femt mole. For more fragile molecules such as tryptamine, tryptophan, phenylalanine and serotonin, the base peak in the mass spectra are characterized by fragment ions formed by losses of the amine side chains. Even with this fragmentation, however, it is possible to achieve sensitivity limits that are many orders of magnitude greater than for secondary ion mass spectrometry, without preparing the samples in special matrices. For serotonin, detection limits of 40 femt moles on the surface of a silicon target are achievable. The results also yield a linear relation between the serotonin base fragment ion intensity and the known surface concentration.

Finally, we will offer a number of suggestions for reducing these detection limits further, and will examine the possibility of utilizing this new technology in submicron molecular imaging.

THE IN SITU CHARACTERIZATION OF MATERIALS USING SECONDARY ION MASS SPECTROMETRY-RECENT ADVANCES

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The SIMS technique has become a highly versatile tool for the chemical characterization of solid surfaces, particularly as additional analytical capabilities have been incorporated in the traditional classes of instruments for dynamic (elemental depth profiling) and static (molecular monolayer) measurements. As outlined in Figure 1, many of the recent advances center on sector field (SF) or time-of-flight (TOF) instruments incorporating imaging or laser post-ionization schemes to improve lateral spatial resolution, selectivity, or sensitivity. As is illustrated by the paper in this symposium by Winograd, the TOF-SIMS instruments have been especially suited to the use of resonant and non-resonant ionization involving pulsed lasers. A commercial SIMS instrument based on these features was recently introduced by Perkin Elmer-Physical Electronics Division known as the SALI (Surface Analysis by Laser Ionization). Paralleling the prior developments in the use of ion microscope or microprobe SF instruments operating in the dynamic SIMS mode (e.g., the Cameca IMS 3-F and IMS 4-F instruments), similar imaging approaches have recently been incorporated in TOF-SIMS instruments operating in the static mode. The presentation by Chakel in this symposium illustrates the combined microscope and microprobe capabilities of a TOF-SIMS instrument recently developed by Charles Evans and Associates. For both SF and TOF instruments, the microscope mode permits the simultaneous stigmatic imaging of areas on the order of 100 μm in diameter with approximately 1 μm lateral resolution. The microprobe sources, such as the field emission liquid metal ion source, provide improved lateral resolution (in the 50 nm range), but are usually restricted to smaller image fields as a consequence of analysis time considerations. Pulsed microprobes utilized in TOF instruments are further constrained by space charge and pulse width considerations that may degrade lateral and mass resolutions, respectively (1). To date, there has been little reported effort to combine both the imaging and post-ionization modes in a given SIMS measurement.

Because of the TOF emphasis of other SIMS presentations in this symposium, I will focus on recent developments in the SF techniques. Of special significance are advances involving three-dimensional (3-D) image depth profiling, incorporating digital image acquisition and processing techniques in SF ion microscopes. This has been a subject of extensive investigation in my research group beginning in the early 1980s (2). In recent years, detection systems generally have utilized microchannelplates (MCP) coupled to either resistive anode encoders (RAE) or phosphor screen/charge coupled device (CCD) assemblies (3,4). We have been particularly interested in various aspects of the CCD approach including: 1) development of intelligent image acquisition systems to optimize the trade-offs between detection sensitivity and spatial resolution, 2) quantification of 3-D data including corrections for detector non-linearities, matrix effects, and interfacial ion yield transients, 3) visualization of 3-D data involving the application of volume rendering techniques, 4) application of pattern recognition techniques to SIMS image data. Image acquisition and quantification issues will be illustrated using applications to dopant characterization in electronic materials (5). Organic applications include the 3-D characterization of spatially modified materials such as patterned arrays of electrically conductive polymer films, or labelled antigenic sites within biological tissues. The use of immuno-gold/silver staining of selective antibodies allows 3-D mapping of antigenic sites within complex biological matrices.

References
Figure 1. Comparison of analytical modes available using sector field and time-of-flight instrumentation for secondary ion mass spectrometry (SIMS) of solid surfaces. Sector field instruments are used extensively for dynamic SIMS involving imaging and/or elemental depth profiling. Time-of-flight techniques are well suited to static SIMS investigations of molecular monolayers, including the incorporation of laser post-ionization techniques and imaging capabilities. (MCP = Microchannelplate, RAE = Resistive Anode Encoder, CCD = Charge Coupled Device, REMPI = Resonant Enhanced Multiphoton Ionization, MPI = Multiphoton Ionization (Non-resonant), SPI = Vacuum Ultraviolet Single Photon Ionization).
ORGANIC SURFACE ANALYSIS AND ION MICROSCOPY WITH TIME-OF-FLIGHT SECONDARY ION MASS SPECTROMETRY (TOF-SIMS)

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Mass spectroscopic analysis of the top monolayers of organic or inorganic surfaces can be achieved using the technique of static SIMS (TOF-SIMS). The low primary ion doses used in static SIMS ($10^{10}$ to $10^{12}$ ions/cm$^2$) are sufficient to generate secondary ions from the surface for mass analysis while minimizing the damage of the surface. The secondary ions formed are often molecular ions or structurally significant fragment ions indicative of the chemical structure of the surface.

A unique configuration of static SIMS instrumentation incorporates a high performance TOF mass spectrometer that provides both high mass resolution and high mass range analysis. The TOF-SIMS instrument can perform mass resolved ion image analysis. With the ion microscope analysis mode, a uniform Cs$^+$ primary ion beam impinges on the entire sample analysis area (typically 60 or 200 micrometer in diameter). Secondary ions thus formed are focused through the TOF mass spectrometer preserving their spatial origin and a magnified ion image is projected onto a position sensitive detector. Lateral distributions can be determined down to resolutions of 1 to 3 micrometer. For higher lateral resolution, an ion microprobe analysis mode is required. With this technique, a microfocused (2000Å beam diameter) Ga$^+$ beam is rastered over the sample surface. A unique feature of this mode is the ability to define custom raster patterns in order to obtain mass spectra from irregularly shaped features or particles. Up to four separate "region of interest" spectra can be acquired simultaneously. A low energy electron charge compensation system permits the analysis of insulating surfaces.

This technique has been used to study the surface of polymeric films, monolayer films, composite materials, treated papers and biological tissues. A representative polymer spectrum for poly (ethylene terephthalate) is shown in Figure 1. The base peak is C$_7$H$_4$O$^+$ at m/z 104. A monomer, dimer and trimer species can be seen at m/z 193, 385 and 577, respectively. The probable ion structures for the peaks labeled with dots in Figure 1 are shown in Scheme 1. Biological analyses including evaluating the relative ion yields and detection limits for biologically active compounds (neurotransmitters and pharmaceuticals) incorporated into different matrices were performed and are presented in Paper MOC 12:15 (C. John, et al.).

Figure 1. Positive ion mass spectrum of poly(ethylene terephthalate).

Scheme 1. Probable ion structures for labeled peaks in Figure 1.

\[
\text{m/z} \quad 76 \quad 104 \quad 149 \quad 193 \quad 341 \quad 385 \quad 577
\]
We are using negative ion photoelectron spectroscopy to study neutral and anionic coordinatively unsaturated transition metal species in the gas phase. Anions are prepared from stable organometallic precursors in a flowing afterglow ion-molecule reactor, and mass-selected by a 90° sector magnet. Use of a sector magnet in place of the Wien filters employed in previous instruments of this type provides a ten-fold improvement in mass resolution. The mass-selected ion beam is intersected by an argon ion laser beam, and the resulting photoelectrons are energy analyzed at a resolution of 5 meV (40 cm^{-1}).

Multiple metal-metal bonding can be examined in its clearest form in the bare transition metal dimers. Chromium dimer, in particular, has been the subject of numerous experimental and theoretical studies. Since the ground state of the Cr atom has a high spin 3d^{5}4s^{1} configuration, a closed shell configuration for Cr$_{2}$ would lead to a formal bond order of six. Consistent with this picture, Cr$_{2}$ is known to have a $^{1}$E$_{g}^{+}$ ground state and a bond length of only 1.6788 Å. However, other results belie the simple picture of a hextuple bond in Cr$_{2}$. For example, the bond dissociation energy (D$_{0}$) of Cr$_{2}$ is only 1.44±0.05 eV (33.2±1.2 kcal/mol), actually lower than the 2.01±0.08 eV bond energy of the singly 4s-4s bonded Cu$_{2}$ molecule. The bonding picture is complicated by the 3-times greater size of the 4s than the 3d orbital in atomic chromium, which may cause the 4s-4s interaction in Cr$_{2}$ to be repulsive at the optimum distance for 3d-3d bonding. Modified GVB calculations by Goodgame and Goddard predict that the ground state of Cr$_{2}$ has a double minimum potential energy curve, dominated by five covalent 3d-3d bonds at short internuclear distance, and by a single 4s-4s bond at long distance.

We report the 488 nm photoelectron spectrum of Cr$_{2}^{−}$. The electron affinity of Cr$_{2}$ is 0.506±0.006 eV. Vibrational levels up to v=6 in the $^{1}$E$_{g}^{+}$ ground state of Cr$_{2}$ fit a Morse potential with $\omega_{e}=479\pm2$ cm$^{-1}$ and $\omega_{e}X_{e}=13.5\pm1.0$ cm$^{-1}$. This anharmonicity constant is unusually large: $\omega_{e}X_{e}$ values for all other neutral homonuclear first row transition metal dimers measured to date fall in the range 1-4 cm$^{-1}$. An excited electronic state of Cr$_{2}$ with a vibrational frequency of 580±20 cm$^{-1}$ is observed 14,240±30 cm$^{-1}$ above the ground state. For Cr$_{2}^{−}$, we obtain $\omega_{e}=470\pm25$ cm$^{-1}$, $\omega_{e}X_{e}=20\pm10$ cm$^{-1}$, and r$_{e}=1.705\pm0.010$ Å.

Perhaps the most intriguing feature of the photoelectron spectrum is the appearance of a weak vibrational progression of Cr$_{2}^{−}$ comprised of more than 12 peaks from 5100 to 6700 cm$^{-1}$ above the 0-0 transition. Surprisingly, the peak spacings are only 130±10 cm$^{-1}$, and they exhibit no detectable anharmonicity! This low-frequency, harmonic vibrational progression displays an intensity profile noticeably more irregular than that expected for the...
The photoelectron spectrum of a diatomic. Furthermore, the intensity profile changes dramatically when the argon ion laser is tuned from 488 nm to any of the other lines strong enough to be used in this experiment. However, the separation between each peak and the 0-0 transition remains unchanged. These observations suggest a one-photon process involving a resonance of the laser with one or more metastable states of the anion. This resonance allows us to probe regions of the Cr$_2$ potential curve which would normally be inaccessible due to negligible Franck-Condon overlap with the anion ground state.

The 130 cm$^{-1}$ vibrational interval observed here is close to the 110 cm$^{-1}$ value predicted by Goodgame and Goddard$^5$ for the singly 4s-4s bonded, "long-bond form" of the Cr$_2$ ground state. However, the 130 cm$^{-1}$ progression appears only 0.6 eV above the 0-0 transition, and displays no detectable anharmonicity over a 0.2 eV range. In contrast, the MGVB calculation$^5$ predicts the 4s-4s well to be bound by only 0.3 eV, and to lie 1.6 eV above the 3d-3d well. In addition, we measure an unusually large anharmonicity ($\omega_0x_0 = 13.5 \pm 1.0$ cm$^{-1}$) for the "short-bond" region of the potential. These results suggest the need for improved calculations of the ground state potential energy curve of Cr$_2$.

(II) Group VI Metal Tricarbonyls

The photoelectron spectra of Cr(CO)$_3^-$, Mo(CO)$_3^-$ and W(CO)$_3^-$ show vibrational activity in the symmetric modes of the C$_3V$ neutral molecules. Although the extra electron is the lone occupant of a degenerate pair of orbitals in the d$^7$ anions, the spectra provide no evidence for a Jahn-Teller distortion. The neutral molecule symmetric CO stretching frequencies are measured to be 2000±10 cm$^{-1}$ in all three species, about 120 cm$^{-1}$ lower than in the stable metal hexacarbonyl complexes. Surprisingly, the spectra also show activity in the MCO bending vibration. Since the active modes in these direct photodetachment spectra are those associated with differences between the equilibrium geometries of the anion and the corresponding neutral molecule, this observation suggests that the MCO bonds are slightly bent in the anions, and possibly also in the neutral molecules. The electron affinities of Cr(CO)$_3$, Mo(CO)$_3$ and W(CO)$_3$ are determined to be 1.36, 1.34 and 1.87 eV, respectively.

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References
Field desorption and laser desorption mass spectrometry have been used to determine the molecular weights of highly branched polymers (dendrimers). These materials are unusual polymers because the synthetic scheme is designed to yield a product having a single molecular weight, rather than a wide distribution of molecular weights. During the synthesis, a branched "core" is modified by the symmetrical addition of linear or branched appendages (Scheme 1). When polymers of this type were first synthesized by Newkome et al., they were dubbed "arborals" because of their tree-like structures (1). The present study involves polymers having a silicon backbone, which have interesting physical properties and a reactive surface that makes them potentially useful synthetic intermediates (2). Electron ionization may be used to determine the molecular weights of the 'core' and 'add-on' reagents, but the volatility of the products is too low to allow EIMS characterization without decomposition. Field desorption ionization has been used to determine molecular weights of first- and second-generation polymers (M, 5-10 kD).

In the electron ionization mass spectra of the add-on blocks and the core units the high-mass ions corresponded to (M-H)\(^+\) and (M-CH\(_3\))\(^+\). Similarly, in the positive-ion field desorption mass spectra, the high mass ions were M\(_a\), (M-H)\(^+\) and (M-CH\(_3\))\(^+\). Some loss of branches also occurred. In the FD mass spectra of the larger compounds, multiply-charged ions were observed as well as abundant singly-charged ions (Fig. 1). The multiply-charged peaks arose by losses of hydrogen, methyl or variously-sized branches, or combinations of these. The most abundant ions in the negative ion field desorption mass spectra were the (M+Cl)\(^+\). In all these spectra, peaks at 74 u above and below the major ions were observed. These reflect the presence of minor components differing in mass by Si(CH\(_3\))\(_2\)O, that are probably result from scrambling that occurs on heating or after long storage.

Because thermal decomposition made it impossible to determine the molecular weights of the third generation products by field desorption, these values (M, 16-20 kD) were determined by laser desorption. LD/TOF mass spectra of the first- and second-generation products were also acquired to provide reference spectra. The laser desorption spectra show cationized molecular ion peaks, some fragmentation via branch loss, and doubly-charged ions (Fig. 2).
Fig. 1. Positive-ion field desorption mass spectrum of the four-branched second generation dendrimer, MW(avg.) 9058.3 (emitter current 5-10 mA, sum of 20 scans).

Fig. 2. Positive-ion laser desorption mass spectrum of the four-branched second-generation dendrimer, MW(avg.) 9058.3 (355 nM, 9 ns pulse, 5 Hz, sum of 50 shots, no matrix).

**Experimental.** Electron ionization mass spectra: MAT 731, V(acc) 8 kV, t:1200 resolution, ion source 180 °C, direct insertion probe, 60-120 °C. Field desorption mass spectra: MS-1 of JEOL HX110/HX110, V(acc) +/- 10 kV, extraction voltage, +/- 2.5 kV, t:1000 resolution, ion source 80°C, carbon emitters on 10 mM tungsten wires. Laser desorption mass spectra: VESTEC 2000, V(acc) 25-30 kV, Lumonics Nd:YAG laser at 355 nM, 9 ns pulse width, repetition rate 5 Hz. For FD and LD, samples were deposited (1 µl) from solutions of the polymer in toluene (t:200).

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There is strong support for a mechanism by which foreign antigen is internalized by an antigen presenting cell and proteolytically processed to generate a number of fragments which are selectively presented by class II major histocompatibility complex (MHC) molecules for recognition by T cells. To assess the role of antigen processing and to circumvent the difficulties in isolating antigenic fragments in vivo we have developed a cell-free processing system. Mass spectrometry has been utilized as a method for rapidly analyzing the proteolytic products of a model antigen, *Staphylococcus aureus* nuclease (Nase), generated under conditions analogous to those present in the in vivo "processing-compartment." Nase was treated with an acidic endoprotease, cathepsin D under varying conditions of time and pH. The resulting digests were (I) characterized by Frit-FAB LC/MS to identify the fragment sequences and (II) assessed for antigenicity using a T cell recognition assay. We have previously identified 5 regions of Nase as T cell determinants in BALB/c mice (I-Ad and I-Eα) (1). In digests carried out over 1, 2, 4 and 18hr, immunogenic fragments were detected as assessed by the recognition of specific T cell hybridomas and the potential fragment sequences were identified solely by LC/MS. Fragments containing the immunodominant (61-80) and minor (112-130) epitopes were identified. Shorter digestion times (0, 15 and 30min) required the use of electrospray ionization to analyze peptides whose molecular weights exceeded 2400 amu (the effective upper mass limit of the Frit-FAB experiment). This latter technique proved effective in generating exact mass assignments for the higher molecular weight peptides, and was corroborated by an exact mass measurement on the intact Nase, producing a Mr of 16,812 +/- 0.002%.  

DETERMINATION OF GLYCOPROTEIN GLYCOTYPES AND GLYCOFORMS BY ESI-MS

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Carbohydrate chains attached to proteins modulate the chemical and biological properties of the resultant conjugate in numerous ways. These glycan chains have been shown to be of three major types, (glycotypes), high mannose, complex, and hybrid. In addition, each chain displays a polymorphic character that has more recently been characterized as a glycoform distribution, (e.g., Man₃₋₉GlcNAc₃₋₉). Thus, glycoproteins are characterized by considerable microheterogeneity and yield, as a signature, an envelope of molecular weights. The nature of this polymorphic distribution can have considerable bearing on product viability and concerns of quality control, especially for those companies involved in protein-based pharmaceuticals. Batch-to-batch monitoring of glycosylated products, in a cost-effective manner, is a challenging and necessary obligation that biotech firms are preparing to undertake.

We have considered this problem of glycoform characterization by proteolysis, glycopeptide isolation, and profiling these fractions using ESI-MS. Reverse phase HPLC fractionates primarily on the basis of peptide structure with perturbations in retention due to glycan residues. Thus, direct characterization of these fractions can provide an unperturbed measure of glycan distribution. In addition to an envelope of glycoforms the mass intervals provide further insight to glycotyep. As an example of this approach Figure 1 is an ESI-MS analysis of three eluting glycopeptide fractions obtained from ovomucoid. The spectra show doubly charged glycopeptides with mass intervals indicating polylactosyl or complex type glycans. Fraction $22$ is a major peak which was followed by fraction $23$. From the spectra it is clear that the separation is one of glycoforms with the peptides identical. In contrast, fraction $24$ indicates a different peptide, however, the glycotyep appears to be identical. This simple and rapid survey of rHPLC fractions greatly facilitates glycoprotein characterization.

Acknowledgements: NIH, NSF, Finnigan-MAT.
Figure 1.

Frac. #22

Frac. #23

Frac. #24

Ba-1

Ba-1'

Ba-2'
CHARACTERISATION OF OLIGOSACCHARIDES AND GLYCOPROTEINS BY MATRIX-ASSISTED LASER DESORPTION MASS SPECTROMETRY (LDMS).

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The pattern of glycosylation is now recognised as being crucial to the biological activity of glycoproteins. However, structural elucidation of the constituent oligosaccharides is extremely difficult because of their complexity. This paper examines the use of LDMS to characterise these compounds.

Samples examined were intact glycoproteins and glycopeptides with molecular weights up to 150 kDa and oligosaccharides containing from 9 to 19 monosaccharide units. All compounds were examined in the underivatised state and, in addition, the oligosaccharides were examined as permethyl and peracetyl derivatives. For recording the spectra, 50 fmole - 10 pmole of the sample in 0.1 µl of water was mixed with the matrix solution (0.5 µl in the centre of the stainless steel target and allowed to dry at room temperature. The matrix consisted of sinapinic acid (10 nmole) for the glycopeptides and 3-amino-4-hydroxybenzoic acid (10 nmole) for the oligosaccharides. These were dissolved in a mixture of acetonitrile (70%) and water (30%) to which had been added 0.1% trifluoroacetic acid. The targets were loaded into a Finnigan Lasermat time-of-flight mass spectrometer and ions were produced with a 337 nm pulsed nitrogen laser with a power density of around 10^6 W/cm².

The spectra of the oligosaccharides, both derivatised and underivatised, were characterised by [M + Na]⁺ ions (Fig. 1) which were often accompanied by [M + K]⁺ ions in low concentration. No fragmentation was observed. Unlike the spectra of proteins, multiply charged and multimeric ions were not evident. These features make the technique ideal for mixture analysis. Mass measurement accuracy was usually to within 0.1% using a prerecorded calibration file, or better than 0.5 mass units when an internal calibration standard was present. The most accurate results were obtained with the laser power set to just above that required to produce a signal. With higher powers, adduct formation often caused an increase in measured mass; this effect occurred to a similar extent with all similar compounds in a mixture.

There appeared to be little correlation between sample loading and recorded peak intensity. This was the result both of the non-homogeneous drying of the slide and to saturation of the signal at sample loadings in excess of about 30 pmole. Peak intensities varied considerably with successive shots but more accurate values of the intensity could be obtained by summing up to about 20 shots. Sugars with similar structures gave similar responses allowing an internal standard to be used for quantitative measurements. Under these conditions it was found that very accurate quantitative measurements could be made over a wide concentration range as the behaviour of the internal standard exactly reflected that of the sample, both in peak intensity and in adduct formation. Fig. 2 shows a calibration curve for a biantennary oligosaccharide (coded NA-2, [M + Na]⁺ = 1664.5) containing nine monosaccharide residues, using its fucosylated analogue as the internal standard, over the range 3 - 125 pmole. Derivatisation as either the peracetyl or permethyl derivative gave similar results although the sensitivity was increased by about a factor of about ten. Sialylated sugars (underivatised) gave lower responses than neutral sugars and tended to form adducts containing multiple sodium atoms.
Glycopeptides gave good responses, but again tended to form complex adducts with alkali metals, particularly at high concentration. Adduct formation also increased with laser power. Fig. 3 shows plots at two laser powers of measured mass against sample concentration for human transferrin oligosaccharide (4134.5 Da) in a matrix consisting of sinapinic acid to which had been added a low concentration of sodium iodide in order to encourage \([M + Na]^+\) formation. The graph shows a linear correlation between measured mass and peak intensity. The y intercept corresponds to the mass of the \([M + Na]^+\) ion showing that the most accurate mass measurements are obtained at low sample loading. The use of an internal calibration compound with more intense peaks ensures high mass measurement accuracy.

As an example of the application of the technique to a biological sample, Fig. 4 shows the spectrum of bovine pancreatic ribonuclease. The peak at 13,683 Da corresponds to the unglycosylated protein and the peak centred around 14,000 Da appears to be a photochemically produced adduct with the matrix. The other cluster of peaks starting at 14,900 Da correspond to glycopeptides containing the sugars (GlcNac)_2(Man)_7 at Asn-34. Following hydrazinolysis and reacetylation, the released sugars were re-examined by LDMS and gave the spectrum shown in Fig. 5. The ratios of these peaks agreed to within ±1% of those measured by three other, non-mass spectrometric techniques.

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**Fig. 2. Calibration curve for a biantennary oligosaccharide**

**Fig. 3. Variation in measured mass with sample concentration for a glycopeptide.**

**Fig. 4. LDMS spectrum of ribonuclease**

**Fig. 5. LDMS spectrum of high-mannose sugars released from ribonuclease.**
Carcinogenic polycyclic aromatic hydrocarbons such as benzo(a)pyrene are known to bind covalently to DNA in vivo following metabolic activation. Projected strategies for assessing individual exposure and risk require detection and identification of these adducts at environmentally induced levels. To this end we have constructed a time-of-flight mass spectrometer and are investigating both pulsed liquid secondary ion mass spectrometry (LSIMS) using a liquid metal ion (LMI) source and matrix assisted laser desorption/ionization with regards to their sensitivity in this analysis.

Details of the liquid metal ion LSIMS ionization source have been described previously (1). Briefly, a liquid metal ion column directs a focused, 0.1-10 nA, beam of 0-25 keV positive ions onto an area of ≤10 μm in diameter. Samples are deposited on a target wire (~25 μm diameter) and the primary beam is swept across it. The sample droplets are 100-300 μm in diameter. In this way the secondary ionization event is defined in time by the sweep rate and the target diameter. Acceleration voltages of 8-10 kV are typically used. Post acceleration up to 10 kV is possible.

Alternately, the third harmonic of a Nd-YAG laser is used to deliver 10 MW/cm² pulses of 355 nm photons onto a target area of ~50 μm diameter. Samples are deposited onto the end of a 2 mm diameter sample probe. An acceleration voltage of 15 kV is used.

The model compound used, 7,8,9-trihydroxy-10-(N²-deoxyguanosyl)-7,8,9,10-tetrahydrobenzo(a)pyrene (dG-BaPDE, MW 569), was synthesized from the ±-anti-7,8-diol-9,10-epoxide metabolite of BaP and calf thymus DNA. The adduct was isolated chromatographically following enzymatic digestion of the intact adducted DNA.

Preliminary experiments were performed with the LMI source and wire sample probe fitted onto a Kratos MS-50 double focusing sector instrument. No dG-BaPDE peaks were observed from 140 pmoles of sample until the glycerol matrix was acidified with 5% trifluoroacetic acid (TFA). The molecular ion region was dominated sodium adduct ions (Fig. 1a). Furthermore, peaks corresponding to the aglycone fragment, loss of the deoxyribose moiety, can be seen for both the mono- and di-sodiated molecular ions, (Fig. 2). Following the reported use of p-toluenesulfonic acid (p-TSA) to suppress metal adduct ions in FAB analysis of nucleotides (2), 0.2 N p-TSA was used to acidify the matrix and significantly decreased sodiation of the molecular ion (Fig. 1b).

The LMI-LSIMS source on the time-of-flight mass analyzer produced useful spectrum from 65 pmoles dG-BaPDE in glycerol/p-TSA. A signal to background of ~5:1 for the (M+H)+ ion as well as observable aglycone fragment can be seen (Fig. 3).

The use of cinnamic acid derivatives in matrix enhanced laser desorption has been shown to produce high quality spectra of proteins at 355 nm (3). Despite the complexity of the matrix spectra in the low mass range and the relatively poor resolution of the instrument at this stage of development, we have observed peaks corresponding to (M+H)+ and (AH₂)+ with good signal to background from 1.5 pmoles of dG-BaPDE (Fig. 4).

Acknowledgement: This research was supported by DOE Grant No. DE-FG06-88ER60696.

References:
Confirmation of the structure of recombinant proteins is especially important for drugs which are approved for clinical use. Currently, biotechnology companies use peptide mapping with UV detection to monitor recombinant products. Laser Desorption Mass Spectrometry (LD-MS) in conjunction with narrow bore HPLC and chemical and enzymatic degradation provided a fast and sensitive method to confirm the primary structure of Interleukin-2 using only 10 μg of starting material.

**Summary of Results:**

* The native material is homogeneous (but may be a dimer in solution)
* The DNA derived sequence can be verified with high specificity
* Methionine is present at the amino terminus; it seems partially oxidised
* There is no glycosylation at Thr 3
* There are 3 cysteines
* The disulfide bond is between Cys 58 and Cys 105
* The disulfide bond becomes scrambled under tryptic digest conditions
CNBr Digest of Interleukin-2
Main spectrum: Sinapinic acid matrix
Inset: Dihydroxybenzoic acid matrix

Native Interleukin-2
CNBr digest, HPLC fraction 15
Sinapinic acid matrix

After DTT
Before DTT
APPLICATION OF TANDEM MASS SPECTROMETRY TO THE ELUCIDATION OF THE STRUCTURES OF CARCINOGEN-NUCLEOSIDE ADDUCTS: IDENTIFICATION OF CHARACTERISTIC FRAGMENTATION PATHWAYS

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National Center for Toxicological Research, Jefferson, AR 72079, and University of Arkansas at Little Rock, Little Rock AR, 72204.

We have developed an analytical strategy based on FAB ionization and tandem mass spectrometry for the analysis of nanogram to picogram levels of unknown carcinogen nucleoside adducts. This strategy involves: (1) derivatization with TMS groups to increase the analyte signal levels using FAB ionization; (2) the use of constant neutral loss scans to detect selectively the derivatized modified nucleosides; and (3) the use of daughter ion scans to probe the structure of the carcinogen modified base moiety (BH$_2^+$ ion). We have presented preliminary data regarding the feasibility of the first two elements of our analytical approach. Specifically, we have demonstrated that FAB is a sensitive desorption ionization methodology for the TMS derivatives of arylamine modified nucleosides (1), and that constant neutral loss scans can be utilized to identify the protonated molecules of TMS derivatives of carcinogen nucleoside adducts (2). Additional work using constant neutral loss scans, selected reaction monitoring, and isotope dilution methodologies for the analysis of low picogram levels of adducts is presented elsewhere (3). Unfortunately only limited evidence regarding adduct structures has thus far been obtained. The objective of this work is to demonstrate that tandem mass spectrometry can be used to obtain specific data regarding the structures of the adducts detected using the TMS derivatization and constant neutral loss scanning strategy described above.

Tandem mass spectrometry provided little additional structure-specific information when the protonated molecules were mass selected for MS/MS studies. Furthermore, with TMS derivatives, uncertainty regarding the location of the TMS groups, and the existence of isomeric TMS derivatives caused additional problems. However, significant structure specific fragmentation was observed from collisionally activated BH$_2^+$ ions. Fortunately, except for the tris-TMS derivatives, the protonated molecules from all of the derivatized nucleosides showed principally underivatized BH$_2^+$ fragment ions.

Model C-8 substituted deoxyguanosinyl [N-(deoxyguanosin-8-y1)-4-aminobiphenyl (dG-C8-ABP) and N-deoxyguanosin-8-yl]-2-aminofluorene (dG-C8-AF)], and C-8 substituted deoxyadenosinyl [N-(deoxyadenosin-8-y1)-4-aminobiphenyl (dA-C8-ABP)] adducts gave different and characteristic fragment ions and neutral losses using low energy MS/MS with a triple quadrupole instrument. Nine specific neutral losses, attributed to cleavage within the base moiety with retention of charge on the carcinogen containing fragment, were observed with the deoxyguanosine adducts but not with the deoxyadenosine adduct. Four fragments attributed to loss of the carcinogen moiety were also observed only with the deoxyguanosine adducts, whereas five fragment ions were observed with adducts from the same carcinogen and different nucleosides. The latter were attributed to loss of most of the base moiety with retention of the charge on the carcinogen containing fragment. Preliminary data suggest that the BH$_2^+$
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Fragment from other types of adducts (N\textsubscript{2}-substituted for example) also give unique fragment ions after collisional activation (data not shown). All of the fragments listed above (and tabulated below) can be attributed to the loss of even electron species (small neutrals) from the BH\textsubscript{2}\textsuperscript{+} fragment parent ion. This result is consistent with the use of low collision energies (about 50 eV) for the MS/MS experiments.

These data suggest that FAB and tandem mass spectrometry can be used to provide significant structure-specific data for carcinogen modified nucleosides. Furthermore, the tandem mass spectrometry step can be incorporated directly into an overall analytical strategy used to determine experimentally the adduct's molecular weight.

REFERENCES


Table 1. Characteristic cleavages observed in the tandem mass spectra of the FAB produced BH\textsubscript{2}\textsuperscript{+} fragment ions from TMS-derivatized model carcinogen nucleoside adducts.

<table>
<thead>
<tr>
<th>Mass Loss</th>
<th>dG-C8-ABP</th>
<th>dG-C8-AF</th>
<th>dA-C8-ABP</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>70</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>72</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>111</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>112</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>122</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>139</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>151</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>166</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fragment Mass</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>207</td>
<td>+</td>
</tr>
<tr>
<td>195</td>
<td>+</td>
</tr>
<tr>
<td>180</td>
<td>+</td>
</tr>
<tr>
<td>168</td>
<td>+</td>
</tr>
<tr>
<td>153</td>
<td>+</td>
</tr>
<tr>
<td>140</td>
<td>+</td>
</tr>
<tr>
<td>123</td>
<td>+</td>
</tr>
<tr>
<td>112</td>
<td>+</td>
</tr>
<tr>
<td>95</td>
<td>+</td>
</tr>
</tbody>
</table>

Acknowledgement: This work was supported in part by The Center for Indoor Air Research (CIAR #90-002)
GCMS STUDY OF GLUCOSE METABOLISM IN MAMMALIAN CELLS

Yong Y. Lin, Bruce M. Gillum and Charles E. Wright
NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314

INTRODUCTION:

The method utilizing stable isotope and GCMS has been developed for the study of glucose metabolism in mammalian cells. Uniformly labeled \(^{13}\)C\(_6\)-glucose was used to study its metabolism via pyruvate dehydrogenase complex and TCA cycle. By the GCMS-SIM analysis of the glucose metabolites and their isotopomers, the glycolysis, glycogenolysis, and glyconeogenesis in the cells can be estimated. \(^{1-13}\)C-glucose was used to examine the formation of \(^{13}\)C-lactic acid and \(^{12}\)C-lactic acid, from which the metabolic flux through the pentose phosphate pathway can be calculated. The developed method was used to examine the glucose metabolism in cultured human cell lines.

METHODS:

Cultured human fibroblast and lymphoblastoid cells were incubated with 10 mM \([1-^{13}\)C\(_6\)]-glucose (for pentose phosphate pathway study) or 10 mM \([U-^{13}\)C\(_6\)]-glucose (for glycolysis and glyconeogenesis study). The resultant metabolites; glucose, lactic acid, aspartic acid, glutamic acid, and \(\gamma\)-aminobutyric acid were analyzed for their \(^{13}\)C-isotope distributions. Volatile derivatives of the metabolites were made as the following: glucose to the aldonitrile pentaacetate, lactic acid to 2-heptafluorobutoxy-propionyl propylamide, and amino acids to their N-trifluoroacetyl-butyl esters.

GCMS-SIM analysis were performed in CH\(_4\)CI(for glucose and lactic acid derivatives) and in NH\(_3\)Cl(for amino acid derivatives) by monitoring the base peaks showing in the following table:

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>CH(_4)CI (m/z)</th>
<th>NH(_3)Cl (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose ((^{13})C(_{0,1,2,3,4,5,6}))</td>
<td>328,329,330,331</td>
<td></td>
</tr>
<tr>
<td></td>
<td>332,333,334</td>
<td></td>
</tr>
<tr>
<td>Glucose ((^{13})C(_{0,1,2,3}))</td>
<td>328,329,330,331</td>
<td></td>
</tr>
<tr>
<td>Lactic acid ((^{13})C(_{0,1,2,3}))</td>
<td>328,329,330,331</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid ((^{13})C(_{0,1,2,3,4}))</td>
<td>342,343,344,345,346</td>
<td></td>
</tr>
<tr>
<td>Glutamic acid ((^{13})C(_{0,1,2,3,4}))</td>
<td>356,357,358,359,360,361</td>
<td></td>
</tr>
<tr>
<td>GABA ((^{13})C(_{0,1,2,3,4}))</td>
<td>273,274,275,276,277</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS:

Metabolism of \([1-^{13}\)C\(_6\)]-glucose in the fibroblast and lymphoblastoid cell lines obtained from Alzheimer disease (AD) and their age-matched controls were examined. The glycolysis of \([1-^{13}\)C\(_6\)]-glucose via pentose phosphate pathway will lose the \(^1\)C-carbon and result in production of non-labeled \(^{12}\)C-lactic acid, while the glycolysis by direct rout will retain the \(^{13}\)C-carbon in the lactic acid; therefore, the flux to the pentose phosphate pathway can be estimated from the ratio of \(^{12}\)C/\(^{13}\)C in the lactic acid (1). The results (Table 1) indicates that the flux to the pentose phosphate pathway is significantly decreased in fibroblast cells of AD (FA-5 and FA-6). The increase in the flux to pentose phosphate pathway can be achieved by the addition of an artificial electron acceptor, phenazinemethosulfate, to the system (cell line FN-6a).

Metabolism of \([U-^{13}\)C\(_6\)]-glucose in the above cell lines were studied, and the abundance of the
isotopomers in the resultant glucose and lactic acid were shown in Table 2. Analysis of the dilution and distribution of the isotopomers in glucose and lactic acid reveals that the glycolysis of the exogenous glucose appears to be the only metabolic activity observed in the cells, since little or no isotopic dilution was observed with $^{13}$C$_1$ glucose or with $^{13}$C$_3$ lactic acid. In addition, little or no recycling of glucose from gluconeogenesis is indicated, since no significant metabolic enrichment could be observed with $^{13}$C$_1$, $^{12}$C$_2$, and $^{13}$C$_3$ glucose (2).

**Table 1:** 11-$^{13}$C glucose metabolism in cell cultures

<table>
<thead>
<tr>
<th>Cell lines*</th>
<th>$^{13}$C-lactic acid(mM)</th>
<th>$^{12}$C-lactic acid(mM)</th>
<th>$^{13}$C/$^{12}$C ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA-5</td>
<td>0.91</td>
<td>2.00</td>
<td>0.46</td>
</tr>
<tr>
<td>FA-6</td>
<td>1.28</td>
<td>2.17</td>
<td>0.58</td>
</tr>
<tr>
<td>FN-5</td>
<td>0.53</td>
<td>1.82</td>
<td>0.29</td>
</tr>
<tr>
<td>FN-6</td>
<td>0.64</td>
<td>2.08</td>
<td>0.31</td>
</tr>
<tr>
<td>FN-6a**</td>
<td>0.36</td>
<td>2.24</td>
<td>0.16</td>
</tr>
<tr>
<td>LA-5</td>
<td>2.52</td>
<td>3.50</td>
<td>0.72</td>
</tr>
<tr>
<td>LA-6</td>
<td>2.34</td>
<td>3.59</td>
<td>0.65</td>
</tr>
<tr>
<td>LA-7</td>
<td>1.29</td>
<td>1.92</td>
<td>0.67</td>
</tr>
<tr>
<td>LN-5</td>
<td>1.95</td>
<td>2.99</td>
<td>0.65</td>
</tr>
<tr>
<td>LN-6</td>
<td>2.23</td>
<td>3.24</td>
<td>0.69</td>
</tr>
<tr>
<td>LN-7</td>
<td>1.12</td>
<td>1.92</td>
<td>0.62</td>
</tr>
</tbody>
</table>

* Fibroblasts FA-5 and FA-6 are from AD patients, and FN-5 and FN-6 are from the age-matched controls; lymphoblastoid lines LA-5,6 and 7 are from AD patients, and LN-5,6 and 7 are from the age-matched controls.

**Table 2:** Mass isotopomer Analysis of Glucose Metabolism (% abundance)

<table>
<thead>
<tr>
<th></th>
<th>Inc. Med.</th>
<th>C$_0$</th>
<th>C$_1$</th>
<th>C$_2$</th>
<th>C$_3$</th>
<th>C$_4$</th>
<th>C$_5$</th>
<th>C$_6$</th>
<th>C$_7$</th>
<th>C$_8$</th>
</tr>
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<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td>57</td>
<td>8.0</td>
<td>1.8</td>
<td>0.65</td>
<td>1.7</td>
<td>5.8</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoblastoid</td>
<td></td>
<td>61</td>
<td>8.9</td>
<td>1.6</td>
<td>0.41</td>
<td>0.28</td>
<td>2.0</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>59</td>
<td>9.0</td>
<td>1.6</td>
<td>0.29</td>
<td>0.43</td>
<td>2.8</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>59</td>
<td>9.0</td>
<td>1.6</td>
<td>0.29</td>
<td>0.43</td>
<td>2.8</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>46</td>
<td>12.0</td>
<td>2.1</td>
<td>0.23</td>
<td>0.54</td>
<td>3.3</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>59</td>
<td>8.9</td>
<td>1.6</td>
<td>0.40</td>
<td>0.34</td>
<td>3.1</td>
<td>27</td>
<td></td>
<td></td>
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</tbody>
</table>

REFERENCES:
INTRODUCTION

Epidemiological studies suggest a role for diet in the etiology of many human cancers. Among the heterocyclic amine food mutagens thus far isolated, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) has been identified in fried beef and fish at levels up to 15 µg/kg (1). The compound is mutagenic in bacteria (2) and has been shown to cause lymphomas when administered in the diet to mice (3). To assess possible human exposure to this compound, we have developed a GC/MS method for measuring PhIP in urine. This study reports on the application of this method to a preliminary study in rats.

EXPERIMENTAL

Internal Standard  A d5-labelled PhIP internal standard was prepared by reaction of d5-phenyl alanine and creatinine, with subsequent purification by HPLC. Methane Negative Ion Chemical Ionization (NICI) mass spectra of the bis-pentafluorobenzyl (BPFB) derivatives of d5-labelled and unlabelled PhIP, are shown in Fig. 1.

![Figure 1: Methane Negative Ion Chemical Ionization mass spectra of A) BPFB-PhIP, and B) BPFB-PhIP-d5](image)

Treatment of Rats with PhIP  Ten 7-week old male Sprague Dawley rats (average weight 211 g) were divided into two dose groups and placed in metabolic cages. One group received a single oral dose of 50 µg PhIP (-250 µg/kg bw) dissolved in 0.5 ml H2O containing 15 µl DMSO and 2 µl 0.1N HCl to aid solubility. The control group received only solvent. Urine and feces were collected every 24 h for 4 days and stored at -80°C.

Extraction of PhIP from Urine  PhIP was extracted from urine using a modification of a procedure described by Murray et al. (4) for MelQx in urine. After addition of d5-PhIP internal standard, PhIP was extracted from 10 to 300 µl aliquots (0.5 or 2% of 24 h urine volumes) of urine (pH 9) with ethyl acetate, extracted into 0.1N HCl and finally extracted from basic aqueous solution with ethyl acetate. Samples were then taken to dryness with a speed vac evaporator.

Formation of BPFB-derivatives  To obtain volatile, electron-capturing derivatives, the PhIP residues were treated for 1 h at 30°C with 400 µl of a 5% solution of pentafluorobenzyl bromide in acetonitrile and 100 µl of diisopropylamine (4). After reduction to dryness under a stream of N2 at 30°C, the residue was dissolved in 0.1N HCl and cleaned up with hexane before extraction from basic aqueous solution with ethyl acetate.
Gas chromatography/mass spectrometry (GC/MS) analyses were carried out on a Hewlett Packard (HP) 5890 gas chromatograph coupled through a heated interface (300°C) to a HP 5988A mass spectrometer. Chromatographic separation was achieved on a HP-1 fused silica capillary column (12 m x 0.2 mm id). After splitless injection at 180°C, the column oven was heated to 290°C at 30°C/min and then at 10°C/min to 320°C. The mass spectrometer was operated in the negative ion chemical ionization mode with a methane source pressure of about 1 Torr and source temperature of 250°C. Quantification was by selected ion monitoring of the (M-PFB)- ions of the BPFB derivatives of PhIP (m/z 403) and d5-PhIP (m/z 408) as shown in Fig. 2.

**Figure 2:**

Selected Ion Monitoring Chromatograms for

A) BPFB-PhIP-d5 (int std), and

B) BPFB-PhIP in the urine of a rat treated with 50 μg PhIP.

**Figure 3:**

Mean daily excretion of PhIP in the urine of 5 rats treated with a single oral dose of 50 μg PhIP. Standard deviation is expressed by error bars.

**RESULTS AND DISCUSSION**

Figure 3 shows the mean urinary excretion of PhIP for 5 rats treated with a single 50 μg oral dose of PhIP. About 0.6% of the total dose was excreted in the first four days after treatment. About 55% of this amount was excreted during the first 24 h. For rat urine, the method has a detection limit of about 2 pg (~1 ng PhIP excreted/24 h) and should be able to detect a single oral dose of ~200 ng PhIP in rats.

Work is underway to improve the sensitivity of the method using immunosaffinity cleanup and to extend the method to matrices other than urine.

**REFERENCES**

(2) Esumi et al., (1989) Jpn J Cancer Res, 80, 1176-1178
(3) Felton et al., (1986) Environmental Health Perspectives, 67, 17-24
ROOT ESSENTIAL OIL COMPOSITION FROM ARISTOLOCHIA ASCLEPIADIFOLIA (ARISTOLOCHIACEAE)

George R. Waller, Oklahoma State University, Stillwater OK 74078, Lorenzo Sagrero-Nieves, Instituto de Ciencias Basicas, Xalapa 91000, Veracruz, Mexico, and Richard P. Sgaramello, International Flavor and Fragrances, Union Beach NJ 07735

The genus Aristolochia, a member of the Aristolochiaceae (1) is distributed in tropical and subtropical regions of the world. Aristolochia asclepiadifolia Brandegee is an endemic plant of Veracruz State, Mexico. The species is a vine 30-10 m long and is locally called "guaco". The root is very aromatic and some people of the region use alcoholic infusions to combat the venoms of scorpions and snakes and for dysentery. The work reported describes the chemical composition of the essential oil of the root of Aristolochia asclepiadifolia, in which 38-83 compounds were identified in 1986-1990.

The vegetal material (root) was collected from random lianas (vines) in the final fruiting stage growing near Actopan, Veracruz in June of each year. The plant is perennial and a voucher specimen is deposited at Instituto de Ecologia Herbarium, Xalapa, Veracruz, Mexico. Approximately 600 g of the ground root was subjected to steam distillation for 6 h in an all-glass system containing no stopcock grease. The distillate was saturated with sodium chloride (Baker, certified reagent grade) and extracted with ethyl ether, ACS-certified reagent grade. The extract was dried over anhydrous magnesium sulfate, filtered, and freed of ether and the essential oil stored at 0°C until analyzed.

Capillary gas chromatography (CGC/MS/DA) analysis was carried out with a Kratos MS-50 mass spectrometer in the electron ionization mode, coupled with a HP 5880 gas chromatograph using a methylsilicone fused silica column 50 m x 0.32 mm. The samples were analyzed using 1.5 μl injections with the splitter turned off and the oven at 50°C, programmed at 2°C/min to 225° and held there for 60 min. The helium flow rate was 1.8 ml/min. Results were acquired and analyzed with a Kratos DS-55 data system (2). Identification was based on comparison of known with unknown spectra, visual interpretation of the fragmentation patterns, and relative retention times are compared to a proprietary IFF standard index (comparable to Kovac's Indices).

Preliminary study of essential root oil of A. asclepiadifolia reported identification in years 1987 and 1988 of 38 compounds (3) with the presence of vanillin. In years 1989 and 1990 eighty-three compounds were identified; between 170-180 compounds were detected and all of the compounds not identified were sesquiterpenes (MW=204) and sesquiterpene alcohols (MW=220). The freshly prepared essential oil for 1986 possessed the characteristic fragrance evaluation as being: dry — sweet, woody, fir balsam absolute, fresh — mate absolute, fig leaf absolute; comment: — smells like compounds of many absolutes → very pleasant; organoleptic quality — similar to already established natural essential oils. The 1988 oil was similar in fragrance evaluation; however, the 1989-90 essential oils did not have the same fragrance, probably due to the absence of vanillin and like presence of more sesquiterpenes and sesquiterpenoids.

A representative CGC/MS/DA reconstituted tracing of the steam volatiles from the 1989 oil of A. asclepiadifolia is shown in Fig 1. Major compounds were linalool and its oxides, α-bisabolol, and guaiol and isomers; followed by vetiverol, β-selinene, ledol, terpinel (4- and α-plus other isomers and their acetates), pogostol, borneol and its esters are shown in Table 1. Of particular interest was the absence of vanillin, the presence of β-caryophyllene and the identification of more sesquiterpenes and sesquiterpenoids in the 1989-90 oils.

The root essential oil from A. asclepiadifolia is shown on a yearly basis and we are unable to pinpoint the reason. Of particular interest was the ability to biosynthesize vanillin and the lack of enzymes required for its production was noteworthy. There are probably a number of factors involved such as genetics, environmental, or other factors unknown.

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Figure 1. Reconstituted total ion current mass chromatogram of essential oil of Aristolochia asclepiadifolia collected in June 1989.

Table 1. Major Compounds Identified in Essential Oil of A. asclepiadifolia

<table>
<thead>
<tr>
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Metabolites of aromatic compounds in bile of fish exposed to weathered crude oil

Donald W. Brown, Margaret M. Krahn, Douglas G. Burrows, Tracy K. Collier, Catherine A. Wigren, Gina M. Ylitalo, Sin-Lam Chan, and Usha Varanasi

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Northwest Fisheries Science Center
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The Exxon Valdez spilled about 11 million gallons of Prudhoe Bay crude oil (PBCO) into Prince William Sound (PWS), AK, in March, 1989. Aromatic hydrocarbons and heterocyclic compounds such as dibenzothiophenes and their alkyl substituted homologs are abundant in PBCO. We wanted to determine if fish were exposed to these aromatic compounds (ACs) as a result of the spill.

Studies have shown that fish take up ACs from their environment. ACs are readily metabolized and the metabolites are excreted in bile. Our goal was to determine specific metabolites of petroleum-related ACs in bile as definitive evidence of exposure. A halibut was injected with weathered PBCO collected from PWS 11 days following the spill to serve as a positive control in the determination of oil exposure in feral fish. Bile was collected 48 hrs post injection, and the metabolites in 100 μL of bile were hydrolyzed using β-glucuronidase with sulfatase activity. The hydrolyzed (non-conjugated) metabolites were extracted into dichloromethane and the extract was cleaned up by HPLC size exclusion cleanup (Krahn et al., J. Chromatogr. 437:161-175, 1988). The metabolite fraction was analyzed by GC-MS using a 30 m DB-5 capillary column. Three μL were injected splitless at 80°C; the split valve opened after 0.5 min; the oven programmed @ 4°C/min to 300°C; 45-450 amu scanned per sec.

More than 150 metabolites were identified as alcohols or phenols in the PBCO-injected halibut (Table 1); however, a solvent-injected halibut had low levels of a few metabolites. An ion chromatogram of the phenanthrene metabolites from the bile is shown in Figure 1. Spectra of 9-anthracenemethanol standard and of C1-, C2-, and C3-substituted phenanthrene-OHs (PHN-OH) from the bile are shown in Figure 2.

This work provided a reference pattern for the successful determination of metabolites of petroleum-related ACs in bile samples from fish captured in PWS (Krahn et al. in preparation).

Figure 1.
Ion chromatograms showing the C1-, C2-, and C3- PHN-OH metabolites in bile from halibut.
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Table 1.
Hydrolyzed metabolites of aromatic compounds from bile of halibut exposed to Prudhoe Bay crude oil.

Abbreviations: C1- for methyl, C2- for dimethyl or ethyl, C3- for propyl, ethylmethyl, or trimethyl; -OH for the phenol or alcohol of the compound; NPH for naphthalene, FLU for fluorene, DBF for dibenzofuran, DBT for dibenzothiophene, PHN for phenanthrene/anthracene, FLA for fluoranthene/pyrene, and BAA for benz[a]anthracene/chryseene.

<table>
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<th>Name of metabolite</th>
<th>Peaks present</th>
<th>Mass (m/z)</th>
<th>Name of metabolite</th>
<th>Peaks present</th>
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<td>C1-FLU-OHs</td>
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<td>C3-FLU-OHs</td>
<td>11</td>
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<td>FLA-OHs</td>
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Figure 2.
Spectra of 9-anthracenemethanol standard and of three hydrolyzed metabolites from bile of halibut that had been exposed to PBCO.
ANALYSIS OF AN EGYPTIAN MUMMY RESIN BY FAST ATOM BOMBARDMENT
MASS SPECTROMETRY AND GC/MS.

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University of Illinois at Urbana-Champaign, Urbana, IL 61801.

Recently, an Egyptian mummy was acquired by the University of Illinois World Heritage Museum. A portion of the resinous material recovered from its wrappings was subjected to mass spectrometric analysis to determine its composition and to assist in determining the mummy's age. Since the resin sample was expected to contain any of several di- or triterpenoic acids, fast atom bombardment (FAB) was chosen as the mass spectrometric ionization technique due to its ability to produce strong molecular ions with these polar, nonvolatile compounds. The low resolution FAB mass spectrum of the resin acids isolated by base extraction is shown in Figure 1. No peaks were observed above m/z 400, indicating a lack of triterpenoic compounds. The molecular formula obtained from high resolution measurements of peaks m/z 299, 315, and 331 (Table 1) contained twenty carbons, characteristic of diterpenoic acids. The molecular formula of the largest peak, m/z 315, contained one more oxygen and two less hydrogen atoms than the common diterpenoid dehydroabietic acid, 1 (Scheme I), suggesting that this unknown acid may be an oxo derivative. The location of the oxo group was determined to be on C-7 by comparison of analogous FABMS/MS daughter ions of the m/z 315 peak to EI fragments and structures of methyl dehydroabietate reported by Enzell and Wahlberg[1]. The presence of 7-oxodehydroabietic acid, 2, was confirmed by comparing the FABMS/MS spectrum of the methyl ester of the unknown with that of an authentic sample. The m/z 299 and 331 peaks were determined by FABMS/MS to be due to two additional oxidation products of dehydroabietic acid, Δ[double bond]6-dehydroabietic acid, 3, and 15-hydroxy-7-oxodehydroabietic acid, 4. The three peaks at m/z 253, 269, and 285 corresponded to loss of COOH and H from the three parent acids. The presence of these characteristic diterpenoids indicates that the base of the mummy resin is a pine pitch obtained from one or more species of conifers.

A problem appeared in determining the mummy's age in that the decorations on the mummy's wrappings suggest that it is from the Roman period of ancient Egypt (100 - 350 A.D.), while a carbon-14 date of 190 B.C. places the mummy in the Ptolemaic period. GC/MS of the neutral fraction identified a series of n-alkanes with chain lengths of 19 to 33 carbons. The finding of similar amounts of odd and even hydrocarbons as shown in Figure 2 suggests a petroleum product as their source as opposed to an insect or plant wax. The only petroleum product available in Egypt at this time was bitumen, a highly oxidized and weathered asphalt imported from regions surrounding the Dead Sea. The use of bitumen in mummification has often been debated; however, its use in later mummies, such as this mummy from the Roman period, has been documented[2]. Confirmation of the addition of bitumen was obtained by trace metal analysis of the resin sample. The elements vanadium, nickel, and molybdenum are characteristic of petroleum products such as
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...bitumen [2]. The presence of 65.9 ppm vanadium, 33.4 ppm nickel, and 17.4 ppm molybdenum in the sample support the addition of an ancient carbon source such as bitumen and would explain the inconsistent carbon-14 date obtained for this mummy.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Sarah Wisseman of the University of Illinois Program on Ancient Technologies and Archaeological Materials for the mummy resin sample and the invitation to participate in this program; Dr. Curt Beck of the Amber Research Laboratory, Vassar College, for authentic samples of dehydroabietic acid and 7-oxodehydroabietic acid; and the University of Illinois Mass Spectrometry and Microanalytical Laboratories.

Support for this work was provided in part by a grant from the National Institute of General Medical Sciences (GM27029) and in part by the University of Illinois University Scholars Program. M.L.P. would like to thank the Department of Education for fellowship support.

REFERENCES


Table 1. FABMS High Resolution Data

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Scheme I

[Diagram of Scheme I]
A PENTAQUADRUPOLE STUDY ON THE GAS-PHASE REACTIVITY OF ACYLIUM AND OXONIUM IONS AND SOME SULFUR ANALOGS

Marcos N. Eberlin and R. G. Cooks, Purdue University, Department of Chemistry
West Lafayette, IN, 47907.

Acylium and oxonium ions constitute two of the most important classes of gas-phase ions. To explore their chemistry, various acylium ions (a) R=H, CH₃, C₂H₅, n-C₃H₇, Ph, p-CH₃O-Ph, p-NO₂-Ph, OH, OCH₃, Cl, NHCH₂N(CH₃)₂, α-β unsaturated acylium ions (b) R,R'=H, CH₃, i-C₃H₇, and (c), oxonium ions (d) R=H, CH₃, C₂H₅ and (e) X=O and some sulfur analogs (e) X=S, (f) R=H, NH₂, CH₃ and (g) were prepared and mass-selected. Their ion/molecule reactions with isoprene and 1,3-dioxolanes were studied in a pentaquadrupole mass spectrometer (Figure 1). This instrument is particularly useful for studies of ion/molecule reactions since two separated reaction regions are available so that either ion/molecule reactions or collision dissociation can be performed in each region when applying any of the fifteen MS³ scans available.

Acylium ions (a) react with isoprene by two competitive reactions, proton transfer and [4+2*¹] Diels-Alder cycloaddition at the CO triple bond (Scheme 1), as exemplified for the acetyl cation in Figure 2, the cycloaddition reaction leading to m/z 111. The other products are due to proton transfer (m/z 69) and subsequent reactions involving protonated and neutral isoprene. The relative extent of these two reactions is greatly affected by the nature of the substituent group (R). The α-β unsaturated acylium ion (b, R,R'=H) react mainly at the double bond, the adduct having enough internal energy to fragment by CO loss. The C≡O⁺ moiety acts as an olefin activating group. Substitution at the C≡C double bond favors cycloaddition at the C≡O⁺ triple bond. Ion c reacts with isoprene at the C≡N double bond to form an abundant cycloaddition product. This product does not fragment by loss of CO as expected by the low stability of the nitronium ion which would be generated.

Scheme 1

The thioacetyl cation (f, R=CH₃) also reacts predominantly by [4+2⁺] cycloaddition. Contrary to the behavior of oxonium ions, which undergo proton transfer reactions, the sulfonium ions react with isoprene to give mainly the cycloaddition products. Acylium (Figure 3), oxonium, thioacylium and sulfonium ions undergo a reaction with 1,3-dioxolanes which formally corresponds to oxirane addition. The mechanism of reaction for acylium ions is assigned as initial O-acylation of the dioxolane, followed by loss of an aldehyde and recyclization.

The sequential product MS² scan was applied to obtain structural information on the ion/molecule products, which helped both in assigning reaction mechanisms, as well as in characterizing these ions. For instance, the fragmentation to the benzoyl cation (m/z 105) displayed in the MS² spectrum of the cycloadduct of ion e (Figure 4), which occurs in a process involving phenyl migration, allows its identification as the product of reaction at the C=N double bond. Also, the structures of the oxirane addition products in the reaction with 1,3-dioxolanes is revealed by the fragmentation displayed in the MS² spectra. The similarity of the MS² spectra for acylium and thioacylium ions with the CID product spectra of ions h shows that this is the product structure while comparison with the CID product spectrum for O-methylated 1,3-dioxolane indicates that oxonium ions yield a mixture of ions (i) and (j). The reactivity with isoprene and 1,3-dioxolanes allows easy characterization of these important gas-phase ions.
Ions with formally separated radical and charge sites (distonic ions) are of wide current interest. However, the knowledge on the properties of distonic ions is almost exclusively limited to highly excited and short-lived species. For example, thus far the ion-molecule reactions of only three β-distonic ions have been investigated (1-3).

We have used a dual cell FT-ICR mass spectrometer to synthesize and study long-lived distonic ions with one or more heavy atoms between the charge site and the radical site. For all the distonic ions that have a protonated heteroatom, fast proton transfer dominates the bimolecular reactions. These distonic ions behave like the corresponding even-electron ions, i.e., the β-distonic isomer of ionized ethanol shows reactivity similar to that of protonated ethanol, the distonic form of ionized trimethyl triphosphate behaves like protonated trimethyl triphosphate, and the distonic isomer of ionized ethyl acetate behaves like protonated γ-butyrolactone. The distonic ions that are not acids, e.g., the ring-opened form of ionized trimethylene oxide (CH₂CH₂O=CH₂⁺) and the ring-opened form (4) of ionized cyclobutanone (CH₂CH₂CH₂C=O⁺) undergo radical type atom and group abstraction reactions with ketones, aldehydes, H₂S and dimethyl disulfide. Bond formation occurs at the radical site of the ion in these reactions. Evidence for one of the reactions is presented in Figure 2 that compares the CAD spectra of two reference ions (b, c) to that (c) of the product ion from hydrogen atom abstraction from acetone by CH₂CH₂CH₂CO⁺ (Figure 1).

Overall, most of the distonic ions studied are clearly less reactive than their conventional radical cation counterparts. Bimolecular reactions involving neutral reagents with low ionization energies and relatively high proton affinities or low homolytic bond energies (e.g., dimethyl disulfide, cyclohexanone, trimethyl phosphite) proved to be useful in distinguishing distonic ion structures from conventional radical cations. For example, while the unrearranged molecular ions of organic ketones undergo charge exchange at collision rate with dimethyl disulfide, the ring-opened isomer of the molecular ion of cyclobutanone preferentially abstracts a CH₂S-group in a radical type reaction (k⁺/k⁺CH₂S = 0.4), accompanied by slow dissociative electron transfer (Figure 3).

References.

Fig. 1  
Reaction of Ionized Cyclobutane with Acetone

Fig. 2  CAD (30-50 eV)

- Reaction product
  \[ \text{C}_2\text{H}_3\text{O}^+ + \text{C}_2\text{H}_5^+ \]

- a)
  \[ \text{H} + \text{O}^+ \]

- b)
  \[ \text{H} + \text{C}_2\text{H}_3\text{O}^+ \]

- c)
  \[ \text{H} + \text{C}_2\text{H}_3\text{O}^+ \]

Fig. 3  REACTION OF M^+ WITH CH$_3$S-S-CH$_3$ (700 ms, 1 x 10$^{-7}$ torr)

- [M + SCH$_3$]

- a) Reaction product
  \[ \text{CH}_3\text{S-S-CH}_3^{+} \]

- b) Reaction product
  \[ \text{CH}_3\text{S-S-CH}_3^{+} \]

- c) Reaction product
  \[ \text{CH}_3\text{S-S-CH}_3^{+} \]
DISTINGUISHING PROTONATED PROPYLENE OXIDE AND PROTONATED PROPANAL IN FT-ICR

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Department of Chemistry, Purdue University, West Lafayette, IN 47907

The intrinsic stability of protonated epoxides is of wide interest, in part because of the great importance of acid-catalyzed reactions involving opening of an epoxide ring in solution, in biological systems, and in chemical ionization mass spectrometry. The energy barrier of the isomerization of protonated propylene oxide to protonated propanal has been estimated to be very low. Distinguishing these two isomers has not been successful.¹

Multistage mass spectrometry experiments with a dual-cell Fourier transform ion cyclotron resonance device were employed to generate the \([C_3H_7O]^+\) isomers with different amounts of internal energy through the use of a series of reagents ions with various gas-phase acidities. The relative stabilities and the structures of the ions generated in these proton-transfer reactions were examined using energy-resolved collisional activation experiments (Argon, 5-100 eV lab.) and ion-molecule reactions with H₂S.

The energy-resolved mass spectra of the epoxide ion are strongly dependent on the exothermicity of the proton transfer reaction used to generate the ion. Protonated propanal does not show similar sensitivity. Both qualitative and quantitative differences are seen in the data for the different isomers. However, the spectra observed for the epoxide ion with average internal energy over 4.5 kcal/mol were similar to those of the protonated propanal (see Figure). This supports the earlier estimation² that the barrier for ring-opening of protonated propylene oxide is about 5 kcal/mol. The dissociation products of protonated propylene oxide and protonated acetone are similar when only a few activation collisions are employed. This suggests that under these conditions, protonated propylene oxide undergoes ring opening to give an intermediate \(\text{CH}_2\text{CH(OH)}\text{CH}_3\) which is common with protonated acetone. In analogy with earlier results obtained by others on protonated ethylene oxide,³ protonated propylene oxide reacts with H₂S by an exothermic reaction with an activation energy of about 4 kcal/mol. This reaction involves loss of H₂O from the collision complex. Protonated propanal does not react with H₂S.
REFERENCES:

Figure. Energy-resolved mass spectrometry of protonated propylene oxide with various internal energy and protonated propanal with 1.3 kcal/mol internal energy.
Electrophilic aromatic substitution (EAS), unlike nucleophilic substitution, proceeds largely by a single mechanism (1). This mechanism, known generally as the arenium ion mechanism, involves electrophilic attack to yield an arenium ion (sigma complex) followed by the departure of the leaving group in the final step (2). Although most electrophilic aromatic substitutions are thought to proceed via the arenium ion mechanism, the participation of Pi-complex intermediates on the potential energy surface can play a role, though to what extent is still up for debate.

One supposition emerging from this debate is that the transition state for EAS is not a rigidly fixed one always resembling the arenium ion. Instead, the transition state may be earlier on the reaction coordinate and resemble starting materials as an oriented Pi-complex. This was found to be the case for gas-phase EAS of alkylated benzenoid species (3).

Our interest in this mechanistic chemistry has motivated the present study in which the scope is to access whether Pi-complexes play a role in the gas-phase EAS of heteroaromatics species such as thiophene, furan, and pyridine.

MS/MS experiments were performed on a Kratos MS-50 Triple Analyzer of EBE geometry, utilizing a high pressure chemical ionization source to conduct bimolecular reactions in the gas phase. Three types of experiments were performed: (a) the heteroaromatic was reacted with alkyl carbenium ions so as to perform gas-phase EAS, (b) alkyl heteroaromatics were protonated via chemical ionization to form identical mass ions to those formed in the aforementioned experiments so as to make direct comparison, and (c) the alkylated heteroaromatic compounds EI spectra were obtained so as to catalog the fragmentation processes intrinsic to the radical ions. In each of the above type of experiment, the ions of interest were collisionally activated and their MIKES spectra obtained.

The protonated alkyl heteroaromatic ions were found in all cases to differ from the corresponding ions formed via gas-phase EAS, which is unlike the case between protonated alkyl benzenes and their corresponding gas-phase EAS analogs. This is readily explainable in that the CI protonation of alkyl heteroaromatics will most probably protonate at the heteroatom, generating a sigma complex. That the ions formed via gas-phase EAS are different from the protonated alkyl heteroaromatics means either that the sigma complex formed via protonation of the heteroatom is not equivalent to the sigma complex formed in EAS, or that the ions formed in EAS are a composite of sigma complex ions and other ionic contributors.

The ions formed via gas-phase EAS share some of the features of the protonated alkylheteroaromatics, but possess two salient fragmentation processes that are unique: loss of a neutral alkene, and loss of the entire alkyl group as a carbenium ion. These two processes trade off as a function of alkyl group chain length -- the longer the alkyl group the greater the propensity for alkyl carbenium ion loss.

The interpretation of the above two findings draws heavily from previous work. The loss of the alkene is most probably a process intrinsic to a sigma complexed species (3, 4). The loss of the alkyl carbenium ion is most probably intrinsic to a Pi-complex (3). The interplay between the two fragmentation processes is logical in that the extent of Pi-complex formation should be a function of incipient carbenium ion stability. The implication then being that both sigma and Pi-complexes potentially play a role in the gas-phase EAS of heteroaromatic compounds.
THIOPHENE + CARBENIUM ION DISSOCIATION PATHWAYS AS A FUNCTION OF CHAIN LENGTH

A GAS-PHASE STUDY OF THE REACTIONS OF PROPENE AND CYCLOPROPA NE RADICAL CATIONS WITH NEUTRAL ETHYLENE

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Early ion-molecule studies have principally dealt with homogenous (i.e., reaction of a radical cation with its corresponding neutral) systems[1,2]. The goal of this study is to understand the mechanism of gas-phase products formed in mixed systems of small olefin and cycloalkane radical cations with other olefins. These reactions are of particular interest because they may react to form a distonic ion (i.e., charge-radical site separate radical cation).

Three tools were used to obtain kinetic and structural information of the reaction products. First, Fourier Transform Mass Spectrometry (FTMS) coupled with a pulse valve interface was used to determine changes in the reactants and products with respect to time. Second, chemical ionization mass spectrometry was used to determine if a covalent product could be collisionally stabilized. Third, tandem mass spectrometry combined with charge stripping was used to determine the structure of the products formed in a high pressure source.

The FTMS experiment was used to study the kinetics of the reactant and product ions. Ethylene was introduced into the FTMS cell at a constant pressure of 1x10⁻⁷ torr with propene pulsed in at a partial pressure of 2x10⁻⁷ torr. The ionization beam was turned on 100 msec after the pulse event so as to achieve maximum reactant ion population. All ions except for those of propene were ejected 300 msec after ionization in order to eliminate any interfering ion-molecule reactions occurring within the cell. The kinetics were investigated by varying the delay time between the ejection and excitation.

The relative abundance of the propene radical cation (m/z 42) decreased while the m/z 55, 41, and 67 ion abundances all increased from 5 msec to 10 sec (See Figure 1). The m/z 55 and 41 ions are probably second order reaction products with the m/z 67 ion appearing around 2 sec as a higher order reaction product.

Tandem mass spectrometry combined with collisional activation has showed the adduct possesses a significant degree of covalency. When C₃H₆ radical cations from either propene or cyclopropane are reacted with ethylene in separate experiments, a product, C₅H₁₀⁺, is formed which fragments upon collisional activation to give the following structurally significant ions: m/z 69 [M - H]⁺, m/z 55 [M - CH₃]⁺, m/z 42 [M - C₂H₄]⁺, and m/z 41 [M - C₂H₄]⁺ (See Figure 2).

Unfortunately, CAD is not informative in terms of distinguishing the ion-molecule reactions from the appropriate model compounds. Charge stripping has been shown to be more effective than CAD in differentiating C₅H₁₀⁺ isomers[3,4].

The charge-stripping experiments were performed in the narrow scan mode from m/z 30 to m/z 36 with diatomic oxygen as the collision gas. The results correspond nicely with those of Holmes; most of the C₅H₁₀⁺ isomers and the two ion-molecule products are distinguishable (See Figure 3). The propene-ethylene charge-stripping mass spectrum closely resembles that of 2-methyl-2-butene spectrum; the presence of a doubly-charged molecular ion is particularly distinctive. The cyclopropane-ethylene spectrum is similar to that of 1,2-dimethylcyclopropane which also shows a doubly-charged molecular ion.
On the basis of the charge-stripping experiments, we propose that the C$_3$H$_6$ radical cations react with neutral ethylene to form a distonic intermediate that isomerizes by 1,2-H$^+$ and 1,2-H shifts (See Figure 4). The propene-ethylene mechanism is in good agreement with Bower's earlier study of propene-propene[2].

Introduction

Gas phase polymeric ions are formed via consecutive addition-elimination (condensation) reactions between unsaturated parent ions with their precursor compounds. One interesting possibility that has not been explored yet is the study of ionic polymerization within van der waals (vdw) clusters of the monomer molecules. The reaction can be initiated following the ionization of a neutral cluster beam formed in a supersonic expansion. Addition and elimination reactions can take place within the ionized clusters resulting in a product ion distribution which reflects both the stability of the polymeric ions and the kinetics of the reaction.

Experimental

Neutral clusters, generated by pulsed adiabatic expansion in a supersonic cluster beam apparatus, were ionized by EI and subsequently mass analyzed in a quadrupole mass spectrometer.¹

Results

Figure 1 displays a segment of the cluster spectrum which includes ions smaller than the dimer, C_{10}H_{16}^{+}. The ions observed are consistent with their formation by eliminative ion-molecule reactions between isoprene radical cation and neutral isoprene within the clusters. These ions are similar to those observed by Kaschers and Cooks from their ion-molecule reactions after 300 ms reaction time in an ion trap². These ions are the characteristic fragment ions observed in the daughter spectrum of limonene, which is formed by a Diels - Alder reaction of isoprene radical cation with its neutral molecule. A magic number has been observed for C_{14}H_{21}^{+} ion and attributed to a stable cyclic structure which can be derived from a series of ion-molecule consecutive addition-elimination reactions. Our results suggest that cationic polymerization of isoprene proceeds via the formation of the stable dimer C_{10}H_{16}^{+} which, most likely, has a structure similar to limonene and can undergo further addition reactions to generate larger ions.

Figure 2 displays a representative segment of a typical 70 eV EI mass spectrum of a cluster beam of vinyl chloride. The precursor ions C_{2}H_{3}Cl^{+} and C_{2}H_{3}^{+} undergo condensation ion/molecule reactions with neighboring C_{2}H_{3}Cl molecules in the cluster with subsequent elimination of neutral species such as HCl, Cl, CH_{2}Cl and C_{2}H_{2}. We observe that the elimination reactions initiated by C_{2}H_{3}Cl^{+} terminate after three successive steps; each involving elimination of HCl or Cl. The product ions are of the general formula C_{6}H_{k}Cl^{+}, k=9-13. These ions can rearrange to some stable structures and this may account for the lack of further elimination reactions.

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¹ M.S. El-Shall and C. Marks, J. Phys. Chem., in press.
Figure 1

Figure 2
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ISOTOPE EFFECTS FOR BASE-PROMOTED, GAS-PHASE PROTON TRANSFER REACTIONS

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Proton transfer reactions are among the most basic, the most common and the most important of chemical transformations; despite their apparent simplicity we still have much to learn about this most fundamental of all chemical processes. Active interest in understanding the underlying principles of organic proton transfer reactions continues because of efforts being made to develop the theory of elementary chemical processes, because of the resurgence of interest in mechanistic organic chemistry and because of the dynamic role played by proton transfers in biochemical transformations. As organic chemists, we have used the flowing afterglow technique to gain an appreciation of the fundamental issues involved in reaction mechanisms by examining such processes in a solvent-free environment under thermally-equilibrated (300 K) conditions. Recent characterization of the facile production of both acetate and the monoenolate anion from the interaction of hydroxide or fluoride with acetic acid reinforces the idea that we have much yet to learn about proton transfers/proton abstractions in general [1]. Earlier work by Riveras and co-workers [2] on competitive H vs D abstraction from a-d-toluences and by Noest and Nibbering [3] on competitive H vs D abstraction from α,α,α-,δ-δ-acetone, in combination with the acetic acid results, challenged us to assemble a comprehensive picture of the competitive nature of proton transfer reactions for anionic base-promoted processes.

We have carried out a broad survey of the "order-of-magnitude" isotope effects that one will see for a large number of systems. Here, we will concentrate on anion-induced proton abstraction reactions from a binary mixture of acetone and δ-δ-acetone, from α,α'-δ-δ-acetone and from α,α,α,-δ-δ-acetone. Such systems allow us to examine inter- vs intramolecular isotope effects, to examine the intramolecular isotope effects for a 1,3 competitive system and to consider isotope effects for hydrogen vs. deuterium on a common carbon center. It quickly became apparent that the bases used should be classified according to structure, not just according to exothermicity. Data for three classes of anions interacting with the binary mixture of acetones are shown on the next page. The first graph describes localized heteroatomic anions (RX-) and shows that there is essentially no isotope effect when the reaction is reasonably exothermic while the isotope effects rises rather abruptly to a value of ca. 1.7 for a thermoneutral reaction. The second graph is for localized carbanionic bases (symbolized HnC-) and shows that the isotope effects are considerably larger here than in the first case; kH/kD is about 1.5 for exothermic reactions and rises more gradually to a much higher value for thermoneutral reactions. These latter reactions are also a bit slower than those represented in the first graph. The final graph is for delocalized carbanions and is divided into two parts; benzylic anions give an isotope effect of ca. 5-7 while allylic anions give a value in the 1.5-3 range. All-in-all, these data demonstrate the sensitivity of isotope effects as a probe of the detailed nature of proton abstraction reactions and promise to provide considerable insight into the dynamics of these fundamental reactions.

Acknowledgment: This work has been supported by a NSF-PYI Award to JJG and by the ACS-PRF.


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Figures for "Isotope Effects for Base-Promoted, Gas-Phase Proton Transfer Reactions". See text on previous page for details of this graphs.
DETERMINATION OF GAS PHASE BASICITIES OF CARBENES
BY FT/ICR MASS SPECTROMETRY

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For about 20 years, experimental techniques such as ion cyclotron double resonance (ICDR) have made it possible to quantitate the gas-phase thermochemistry of proton transfer reactions. By comparing the solution and gas-phase basicities, one can distinguish the intrinsic molecular basicity from solvation effects. Moreover, heats of formation and bond dissociation energies are in turn helpful in determining molecular stabilities and energetically favorable reaction pathways.

Carbenes, R–C–R', are important short-lived intermediates in condensed-phase organic synthesis, photochemistry, and atmospheric chemistry. For example, cyclopropenylidene is the most abundant hydrocarbon in Interstellar space, and other carbenes have been detected in Interstellar and circumstellar space [2,3]. Although proton affinity (PA) and gas phase basicity (GB) have been determined for a wide variety of organic compounds [1], such data on carbenes have to date been limited to a few chloro- and fluorocarbenes [4-6].

In this abstract, we report the formation and detection in a Fourier transform ion cyclotron resonance (FT/ICR) mass spectrometer of several protonated carbenes, CHRR', produced by electron ionization of neutral precursors provided by Professor M. S. Platz. The electron energy is set just above the appearance potential of the carbonium ion. The ions are then transferred from the source region of a dual ion trap to the analyzer region, where a stored waveform Inverse Fourier transform (SWIFT) rf excitation ejects all ions except the protonated carbene. Since the concentration of gas-phase neutral carbene, CRR', is vanishingly small, the gas-phase basicity, GB = ΔGrx = RT loge Keq, cannot be determined directly from the equilibrium constant, Keq, for the reaction.

\[ \text{CHRR'}^+ + B = \text{CRR'} + \text{BH}^+ \] (1)

In which B is a volatile reference base. It is therefore necessary to "bracket" the gas-phase basicity by reacting the protonated carbene with a series of reference bases of different known basicities, to determine the threshold basicity at which BH' is observed (i.e., the forward reaction shown in Eq. 1). A gaseous neutral reference base is injected into the analyzer region of the chamber by means of a pulsing valve, and allowed to react with the protonated carbene for hundredths to tens of seconds, after which the product ions are excited and detected. If BH' is observed (Eq. 1; Figs. 1,2), then one infers that the basicity of the carbene is lower than that of the reference base. In this way, the gas phase basicities of several carbenes have been determined. In cases for which the reference base exhibits self-protonation, thereby obscuring the confirmation of proton transfer from the carbonium ion, deuterated precursors were employed. Our proton affinities are: dichlorocarbene, 196.1±0.2; dimethoxycarbene, 171.3±3.3; and phenylcarbene, 173.0±1.7 kcal/mole respectively (Fig. 3); the latter two values are still being refined. This work was supported by NIH (GM-31683) and Ohio State U.

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Figure 1. FT/ICR mass spectrum of ionic products of the reaction of phenol with deuteriodichlorocarbene ion. The phenol molecular ion, M⁺, phenol self-protonation ion, (M+H)⁺, and deuterated phenol ion, (M+D)⁺ are observed.

Figure 2. High resolution FT/ICR mass spectrum of m/z = 96 u/e species. Deuteron transfer to phenol (right-hand peak) is clearly distinguishable from the ¹³C isotope peak of self-protonated phenol (left-hand peak).

Figure 3. Proton affinity of dichlorocarbene (large print), determined by pairwise comparisons to each of the listed reference bases. Left: ref. 6. Right: this work.
MASS INDEPENDENT ISOTOPE EFFECTS IN THE FORMATION OF $OJ^+$

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Motivated by relevance to interstellar and upper atmosphere chemistry, the termolecular association reaction:

$$O_2^+ + O_2 \rightarrow Oj^+$$

has been the subject of experimental and theoretical investigations for almost thirty years. In 1963, Curran (1) reported an appearance potential for $Oj^+$ produced in reaction 1 of 16.9± 0.1, almost 5 eV greater than that of ground state $O^+$. On the basis of this observation Curran suggested a role for the metastable $a^4\Pi_u$ electronic state of $Oj^+$ in reaction 1, although no consideration was given to the origin of the unusual requirement of electronic excitation for the production of a cluster ion. Unfortunately, no notice of the $Oj^+$ appearance potential seems to have been taken by other workers in subsequent kinetic studies of $Oj^+$ formation leaving the detailed nature of the apparent electronic energy requirement unknown. Very recently, Smith et. al. (2) observed formation of $Oj^+$ by reaction 1 where the $Oj^+$ was produced by a resonance-enhanced multiphoton ionization (REMPI) scheme which is known to produce only electronic ground state ions. Thus the results of Smith et. al. can be compatible with those of Curran only if REMPI and near threshold electron ionization (EI) produce ion state populations which differ in some critical aspect affecting the kinetics of reaction 1 and this difference is preserved in "thermalizing" collisions of the ions. The present work addresses this question by reexamining the threshold EI production of $Oj^+$ paying particular attention to the production of cluster ions containing a single $^{17}O$ or $^{18}O$ atom from $O_2$ molecules with a natural abundance isotopic distribution.

The intensity $^{32}Oj^+$ and $^{64}Oj^+$ (denoted $I_{32}$ and $I_{64}$ respectively) as a function $E_e$ is shown in Figure 1. As $E_e$ is increased above the $O_2$ threshold, $I_{32}$ initially increases substantially faster than does $I_{64}$ indicating that the simple kinetic description: $d[Oj^+]/dt = k_1[O_2][O_2]$ is not valid at low $E_e$. This is a remarkable result considering that for $E_e < 16$ eV, no electronically excited states of $Oj^+$ can be produced and the vibrationally excited levels of the ground electronic state produced are efficiently relaxed to the $v = 0$ level under our ion source conditions. Further evidence that the ionizing conditions have a significant effect on clustering efficiency is provided by the pronounced increase in $I_{64}/I_{32}$ at $E_e = 19-20$ eV in qualitative agreement with the results of Curran.

Fig. 1 Intensity of $Oj^+$ and $Oj^+$ as a function of ionizing energy.

Fig. 2 Intensity ratios of isotopically substituted $Oj^+$ as a function of ionizing energy.
The intensities of O$_j^+$ ions containing one $^{17}$O (les) or one $^{18}$O (lee) relative to the intensity of the all $^{16}$O containing species ($^{16}$O$_4$) as a function of $E_e$ is shown in Figure 2 where the dotted lines indicate the expected ratios based on natural isotopic abundance. At low $E_e$ reaction 1 shows a strong kinetic isotope effect with the the intensity mixed isotopic O$_j^+$ ions approaching a ten fold enhancement relative to natural abundance expectations near threshold ($E_e \approx 12$ eV). Furthermore the enhancement is mass independent with the $^{16}$O$^3$/$^{16}$O$^4$ ratio always observed to have its natural abundance value. As $E_e$ is increased, the $^{16}$O$^3$/$^{16}$O$^4$ and $^{16}$O$^4$/$^{16}$O$^6$ ratios continually decrease and natural abundance values are observed for $E_e \geq 40$ eV. The most dramatic change in the $^{16}$O$^3$/$^{16}$O$^6$ and $^{16}$O$^6$/$^{16}$O$^8$ ratios occurs near the threshold $O_2^3(a^3\Pi_g)$ production ($E_e \approx 16$ eV) suggesting that the $E_e$ dependence of the kinetic isotope effect in reaction 1 results from the increasing participation of $O_a^3$ originally produced in the $a^3\Pi_g$ as $E_e$ is increased. Phenomenologically, the results indicate a strong kinetic isotope effect favoring mixed isotopic cluster ion formation when the $O_2^3(a^3\Pi_g)$ is produced by $E_1$ directly and little or no kinetic isotope effect when the $O_2^3(a^3\Pi_g)$ arises from relaxation of metastable $O_2(a^3\Sigma_u^-)$.

A fundamental understanding the results requires that the following three points be addressed: (1) A characteristic of the $O_a^3(a^3\Pi_g)$ ions must be identified which can give rise to largely varying state specific values of $k_1$. (2) The effect of this characteristic on $k_1$ must be strongly isotopic dependent. And (3) the population distribution of ions with regard to this characteristic must depend on the details of ion formation and must be largely preserved in a collision environment. Termolecular association reactions are generally considered to consist of two bimolecular energy transfer reactions. Ordinarily all of the reactions are considered to occur on a single Born-Oppenheimer surface correlating with ground state products. However, there is no rigorous reason why this should be so. If, for a particular set of collision conditions, the O$_2^3$/O$_2$ collision preferentially took place on a repulsive surface, then the (O$_j^+$)* collision complex would be very short lived and cluster formation would be inhibited. We have developed a permutation-inversion (PI) symmetry group appropriate for correlating rotational-vibrational-electronic-translational wave functions for O$_2^3$/O$_2$ with the electronic wave functions of (O$_j^+$)*. The specific correlation depends on whether the angular momentum quantum numbers $J^+ - S^+$ for O$_j^+$, N for O$_2$, and L for their relative orbital motion are even or odd as listed below.

<table>
<thead>
<tr>
<th>N</th>
<th>J$^+ - S^+$</th>
<th>L</th>
<th>$\Gamma_{st}(C_{2h})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>odd</td>
<td>even</td>
<td>even</td>
<td>A$_u \oplus B_g$</td>
</tr>
<tr>
<td>odd</td>
<td>even</td>
<td>odd</td>
<td>A$_b$</td>
</tr>
<tr>
<td>odd</td>
<td>odd</td>
<td>even</td>
<td>A$_g$</td>
</tr>
<tr>
<td>odd</td>
<td>odd</td>
<td>odd</td>
<td>B$_u$</td>
</tr>
</tbody>
</table>

Considering that the ground state of O$_j^+$ in C$_{2h}$ symmetry is B$_u$, Table I indicates that indicates that O$_j^+$ ions in a state with $J^+ - S^+ = odd$ having an odd L collision with an O$_2$ molecule will access the ground electronic state of O$_j^+$ and lead to termolecular association more efficiently than any other type of collision. This restriction vanishes when one of the diatoms is isotopically heteronuclear. Due to this symmetry restriction the kinetics of reaction 1 depends critically on isotopic composition and mode of ionization.

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OBSERVATION OF A HIGH ENERGY METASTABLE STATE OF O$_2$ FOLLOWING O$_2$/ALKALI METAL CHARGE EXCHANGE

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The title reaction has been studied previously by several groups$^{1-3}$ using neutralized ion beam techniques which were sensitive only to dissociative processes. In those experiments the primary neutralization products were identified as the $^1\text{P}_0$ Rydberg levels which rapidly predissociate. We have investigated this reaction using a neutralized ion beam technique which is sensitive to both dissociative and nondissociative products. Briefly, ions formed by electron ionization in a high pressure source are accelerated to 5 keV and magnetically mass-resolved. The ions then enter a neutralization chamber containing a few mTorr of metal atoms (Na or K). Both neutrals and ions exit the chamber, and the ions are deflected by a pair of electrostatic plates. The neutral beam, which spreads as species dissociate, travels to a translatable detector, which samples the intensity as a function of the angle ($l(\theta)$ vs $\theta$). Metastable products show up as a spike of intensity centered around $\theta=0$. A typical profile is shown in Figure 1.

Our results for the dissociative products are in complete agreement with previous work. Surprisingly, however, undissociated O$_2$ in a long-lived metastable state was also observed. By measuring the dependence of the normalized intensity of the metastable ($I_m/1^0$) as a function of time after formation, a dissociative lifetime of $\sim$2.8 $\mu$s was obtained (See Figure 2).

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**Figure 1** $l(\theta)$ plotted as a function of $\theta$. O$_2$ from 5:1 Ar:O$_2$ neutralized on K.

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**Figure 2** $\ln$ of normalized metastable intensity as a function of time. O$_2$ from 5:1 Ar:O$_2$ neutralized on K or Na. Slope $=-(\tau)^{-1}$, which gives a lifetime of the metastable, $\tau \sim 2.8$ $\mu$s.
Because the metastable relaxes dissociatively, it cannot be identified with any of the known metastable states of O$_2$, all of which lie below the first dissociation limit. The ion states which could be responsible for the metastable species include O$_2^+$ ($^4\Pi_u$) and O$_2^+$ ($^2\Sigma^+_g, v=1,2$). To examine the role of different ion states in the metastable formation, various mixtures of Ar and O$_2$ were used. The ion source has a resonance time of 0.4 µs, and this allows the ions to undergo quenching reactions before exiting the source; thus by varying the Ar/O$_2$ ratio, we can alter the amount of O$_2^+$ ($^4\Pi_u$) and O$_2^+$ ($^2\Pi_g, v=1,2$) exiting the source. In all cases, the normalized intensity of the metastable remained constant, indicating that the metastable species is not produced through either O$_2^+$ ($^4\Pi_u$) or O$_2^+$ ($^2\Pi_g, v=1,2$). However, these results did not exclude high vibrational states of the ground state. To test the effect of high vibrational excitation in the ion on metastable production, a mixture of Xe and O$_2$ was used. Xe is an excellent quencher of both O$_2^+$ ($^4\Pi_u$) or O$_2^+$ ($^2\Pi_g, v=1,2$), and the charge transfer from Xe$^+$ to O$_2$ is very efficient. These conditions allow us to maintain the O$_2^+$ signal intensity even near threshold for production, while eliminating the ion states we are not interested in. By varying the ionizing electron energy, we can vary the high vibrational excitation. In Figure 3, the normalized metastable intensity is plotted as a function of ionizing electron energy. It is easily seen that high vibrational excitation favors production of the metastable.

Finally, we have investigated the possibility that the metastable species is a high Rydberg level of O$_2$. In order for a Rydberg level to account for the ~ 2.8 µs lifetime, the principle quantum number $n$ must be greater than 10. By attempting to field ionize the metastable species, it was determined that $n$ could not be greater than 14. Thus, for a Rydberg level to explain the observation of a metastable species, $n$ must be between 10 and 14, and not greater than 14. Since these energy levels are so closely spaced, this is extremely unlikely.

No previously observed state of O$_2$ can account for the observations made in this investigation. Currently we are considering Rydberg-valence and valence-valence interactions in the energy region of the first Rydberg levels of O$_2$ as a possible explanation.

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FORMATION AND CHARACTERIZATION OF CROWN ETHER COMPLEXES IN THE GAS PHASE

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The chemistry of macrocycles is important in understanding principles of host-guest chemistry in molecular recognition. In particular, crown ethers and their complexes are useful for modeling biologically relevant transport processes, and enzyme catalysis. Several mass spectrometric analysis of crown ethers and complexes have been reported. The present study compares the mass spectrometric characterization of crown ethers and their gas phase chemistries to several perfluorinated analogues.

Collision induced dissociation of protonated crown ethers, produced by chemical ionization, proceeded by successive losses of ethylene oxide units, and the assumption was made that the fragmented ions retained a cyclic structure. Perfluorinated crown ethers produced an abundant molecular ion in the negative mode and dissociated by the loss of \((C_2F_4O)_n\) units. The loss of two or more ethylene oxide units were more favorable than the unit loss, and the same trend held for the perfluorinated molecules.

Reactions with \(O_2\), \(CO\), and \(CO_2\) were examined since perfluorinated compounds show a high oxygen carrying capacity. Several crown ethers, acyclic ethers, and their perfluorinated analogues were tested and only the perfluorinated crown ethers produced adducts with oxygen. These oxygen adducts fragmented by loss of \((C_2F_4O)\) units, which indicated that these adducts are not just held by electrostatic forces but the bonding is at least as strong as ether linkages. A less abundant fragment series from the loss of the oxygen was also observed (Figure 1).

Some aspects of the negative ion chemistry of crown ethers have been investigated by ion/molecule reactions of halide ions. Abundant halide adducts were produced in the negative chemical ionization mode for a series of ethers, their perfluorinated analogues, and a thio ether \((1,4,7,10,13\text{-pentathiacyclopentadecane})\). The highly electronegative perfluorinated crown ethers only reacted with the fluoride ions, and CAD of the fluoride adduct proceeded only by loss of \((C_2F_4O)\) units. Other ethers examined showed adducts with all halides, however, the iodide and bromide adducts dissociated only to the halide anion. The fluoride adducts showed several crown-related fragment ions and the loss of \(HF\) was also observed. The effects of halide size and gas phase acidity are the properties that are under consideration for rationalization of the CAD behavior. The acidity of fluoride is substantially higher than the acidities of the other halides, so it has the greatest favorability of abstracting a proton from the crown ethers, resulting in \((M-HF)^-\).

Crown ethers have been investigated for alkali metal binding in solutions. The present study has focused on the size selectivity of crown ethers in the gas phase. LSIMS was used to form complexes of crown ethers and alkali metals. Collision induced dissociation of \([\text{alkali metal}]\text{halide}^+\) complexes of several crown ethers revealed that as the cavity size of crowns increase the binding ability for larger alkali metals increase. Another approach used the kinetic method to investigate the binding preference of the crowns with alkali metals. Crown ether adducts of mixed alkali metal dimers were generated in LSIMS mode by appropriate mixing of halide salts. This study suggested that the binding preference of crown ethers was highly correlated with cavity sizes. Table 1 summarizes data for 18-crown-6 and 15-crown-5. The solution stability constants in methanol or water for these crowns show the highest binding toward potassium, then cesium and sodium.

Gas phase recognition of ammonium and related substrates were examined by the formation of adducts of crowns with ammonium hydroxide, methyl amine, methyl hydrazine, and tosyl hydrazine. Collision induced dissociation techniques were used at collision energies of 0.4 keV and 7 keV. At high collision energies these complexes showed fragments indicative of strong interaction of the amine site with the crown ethers. Also, fragmented adducts of \((\text{crown} + 16)^+\) and \((\text{crown} + 32)^+\) were observed with methyl hydrazine and tosyl hydrazine, respectively. These fragmented adducts were not observed at low collision energies; however, the presence of a metastable ion suggested the strong binding of crowns and the amine sites.
ACKNOWLEDGEMENTS

We gratefully acknowledge the MIT Mass Spectrometry Facility and the Robert A. Welch Foundation for generous support of this research. Prof. Brodbelt extends thanks to the ASMS for a Young Investigator Award.
On the basis of current or planned work in several laboratories it is quite clear that the next major frontier in structural mass spectrometry involves the maturing of tandem mass spectrometry and the development of greater sensitivity, specifically the improvement in the signal-to-noise ratio, especially in terms of background noise. Several new instrument designs are in the developmental stage, e.g., multi-sector instruments with spatial array detectors and time-array detectors, Fourier transform ion cyclotron resonance (FTICR), quadrupole ion traps, and sophisticated time-of-flight instruments. Each instrument design possesses specific advantages, in terms of operating parameters and range of experiments, but it is too early to evaluate in detail the specific advantages/disadvantages of a particular instrument concept.

In this paper we describe a new instrument under development in our laboratory. The instrument incorporates a high resolution magnetic sector as MS-I (Kratos MS-50) and a reflectron time-of-flight (TOF) as MS-II. The instrument design is mechanically simple, operator friendly, and takes advantage of the high efficiency of pulsed ionization and time-domain data acquisition. Of particular importance is the applicability of this instrument concept to pulsed ionization/excitation methods such as matrix-assisted laser desorption and multiphoton ionization/dissociation.

The tandem magnetic sector/reflectron time-of-flight (TOF) instrument is shown in Figure 1. The instrument consists of a Kratos MS-50 and a home-built reflectron time-of-flight. The instrument is equipped with a continuous or pulsed 30 keV Cs+ ion gun (Phasor Scientific), an excimer laser (Questek model 2440) for performing pulsed laser desorption and/or photodissociation, and a Laser Science, Inc. cartridge type N2 laser (model VSL 337 and VSL 337ND). Time-of-flight spectra can be obtained by using a time-to-digital converter (TDC) (LeCroy model 4208 TDC), a 350 MHz digital oscilloscope (LeCroy model 9450), or a transient recorder (LeCroy model TR8828D 200 MHz digitizer). All of the TOF data acquisition modules are interfaced to PC/AT computers by using a National Instruments GPIB mainframe card. Event sequenceing for the various experiments is controlled by a EG&G Princeton Applied Research digital delay generator model 9650.

The reflectron TOF currently in use has a total flight path of 0.5 meters and the reflection angle
is approximately zero degrees. The advantages of a colinear reflectron are high ion transmission and high mass resolution in comparison to that obtained with larger (5°-20°) reflecting angles. The colinear arrangement is also convenient for positioning detectors to detect both neutrals and ions. For example, neutrals formed by metastable ion dissociation reactions, collision-induced dissociation (CID), or photodissociation in the region between MS-I and MS-II are detected by a microchannel plate detector (detector #2) positioned behind the reflectron. Ions are detected by the microchannel plate detector (detector #1) positioned at the entrance to the reflectron TOF.

Tandem MS-TOF Instrument

Figure 1.
Introduction

Fast atom bombardment tandem mass spectrometry of lithiated oligosaccharides has been shown to be a useful technique to determine the linkage position of disaccharides and oligosaccharides [1]. The monolithiated species of various selected disaccharides show a distinguishable pattern in the product ion scan [2]. As reported earlier, gentiobiose, the β-1,6 linked disaccharide, shows product ions of m/z 289, 259 and 229 which correspond to two-carbon (C2), three-carbon (C3) and a four-carbon (C4) chain neutral losses of the reducing end. Lactose, the β-1,4 linked disaccharide, shows only a C2 and a much less intensive C4 neutral loss. Laminaribiose, the β-1,3 linked isomer, forms only an ion at m/z 259 corresponding to a C3 neutral loss. The loss of the C3 neutral species producing a product ion at m/z 259 can be explained only if one considers a rearrangement prior to bond cleavage in the open hydroxy-aldehyde form of the reducing end of the disaccharide. The rearrangement consists of keto-enol tautomerization such that the carbonyl function is transposed from C1 to C2. Once the carbonyl group is located at the C2 position one can postulate a six membered ring transition state from which a retro-aldol rearrangement can be invoked thus giving the ion of m/z 259 [3]. Compared to single bond cleavages the steps involved in the rearrangement are clearly more time consuming. The various product ion scans of monolithiated laminaribiose and nigerose were presented and discussed.

Experimental

The instrument used in this work was a VG ZAB2-EQ [3] equipped with collision cells in the 1st, 2nd and 3rd field free region. A Cs+-gun operated at 25 kV was used to bombarded the sample. The acceleration voltage was 8 kV.

The samples were dissolved in water with a small amount of Li2CO3. The mixture was then added to a dithiothreitol/dithioerythreitol matrix (3/1).

Results and discussion

Figure 1 shows the various CID product ion scans of the monolithiated molecular ion of laminaribiose. The B/E scan represents the product ion scan in the 1st field free region. The ion of m/z 259 is absent. The rearrangement has not yet occurred during this short time. In the 2nd field free region scan, MIKE scan, the ion of m/z 259 is present. The ions of m/z 229 and m/z 215 in the B/E and the MIKE scan can be explained by high collision energy induced two bond cleavages. 18O-labelling studies of laminaribiose did show that the C4 neutral loss giving m/z 229 can take place either on the non-reducing or the reducing end of the disaccharides. The 18O-labelling experiments further showed that the C5 neutral loss giving rise to the m/z 215 takes place on the non-reducing end. Because the quadrupole product ion scan represents low energy CID processes, the C4 and the C5 neutral loss never show up in this scan.

Both the β and α 1,3 linked isomers gave the same results. The time frame of the experiment is shown in Figure 2. The time taken for an ion to arrive at a given part of the mass spectrometer is given by equation

\[ t = d \left( \frac{m}{2zeV} \right)^{1/2} \]

\[ \text{d} : \text{distance} \]

\[ m : \text{mass of a single ion} \]

\[ z : \text{charge of the ion} \]

\[ e : \text{elementary charge} \]

\[ V : \text{accelerating voltage} \]

Thus the time for the precursor ion to arrive at the gas cell of the 1st field free region is 2 μs. The precursor ion passes the MIKE cell at 55 μs and it arrives after 75 μs at the quadrupole gas cell. Corresponding to the above discussed product ion scans the rearrangement takes place within 50 μs of flight between the 1st and 2nd field free region. The time needed for the rearrangement in laminaribiose and nigerose is therefore about 50 μs.
Conclusion
The C3 neutral loss in laminaribiose and nigerose involves keto-enol tautomerization to form the carbonyl group in the C2 position in the hydroxy-aldehyde form of the reducing end of the disaccharide. Product ion scans from the 1st, 2nd field free region and in the quadrupole mass analyzer clearly showed that the rearrangement takes place in the gas phase between the 1st and the 2nd field free region of the mass spectrometer.

Figure 1  CID product ion scans of laminaribiose.

Figure 2  Time frame of the experiment

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MATRIX CHEMISTRY AND BACKGROUND IONS IN FAB AND LIQUID SIMS

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FAB MS and FAB MS/MS are established analytical techniques. The success of FAB (or liquid SIMS) for producing molecular weight information belies a significant understanding of the chemical events that occur upon keV particle bombardment. Based on ions observed in the low resolution spectrum of neat glycerol, and subsequent OH⁺ CIMS of irradiated glycerol, Field concluded that keV particle bombardment of glycerol produces new molecular species via recombination of matrix derived radicals (1). This conclusion was substantiated by GCMS analysis of bombarded glycerol (2).

We have used accurate mass measurements to assign elemental compositions, and MS/MS to characterize the "peak at every mass" background ions produced by keV particle bombardment of neat FAB matrices (3). Based on these experiments, we have identified a mechanism which accounts for the major background ions observed in glycerol and other common FAB matrices.

Iodization of the matrix radical recombination species produces parent or precursor background ions. These ions along with H⁺-bound matrix cluster ions lose stable neutrals such as H₂O and H₂CO to produce product background ions at lower mass. These relatively low mass, covalent species produce higher mass background ions by clustering with matrix molecules (3).

The matrix chemistry outlined above has two major analytical implications. The matrix derived free radicals may react with stable analyte derived free radicals, giving rise to additional peaks in the mass spectrum, possibly confusing a qualitative analysis. This could possibly lead one to conclude that the compound is "impure". Secondly, background ions increase the limit of detection for MS/MS and target compound analysis, reducing the appeal of quantitative FAB.

The FAB analysis of the herbicide atrazine illustrates the importance of understanding matrix chemistry. The FAB spectrum of the pure compound in 2-hydroxyethyl disulfide (HEDS, molecular weight = 154) shows abundant analyte specific ions in addition to the protonated atrazine molecular ion at m/z 216 (see figure 1). These ions (marked with a dot) can be accounted for by considering a mechanism based on the coupling of analyte derived radical species with matrix derived radical species.

The top of figure 1 shows a mechanism for the formation of a molecular species with a mass of 257 which upon protonation forms the ion at m/z 258. The proposed structure is supported by the elemental composition determined by accurate mass measurements and the MS/MS spectrum (not shown) obtained on a Kratos MS-50 TA.

Now consider m/z 292. Loss of H⁺ from atrazine produces a radical A₂₋. Combination of A₂₋ with H⁻ produces a molecular species with a mass of 291, which upon protonation produces the ion at m/z 292. Loss of H⁺ from HEDS produces a matrix radical H₁₁₋. Combination of H₁₁₋ with the atrazine A₁₉₀ radical produces a molecular species with a mass of 333, which is protonated to yield the ion at m/z 334.

The ion at m/z 182 corresponds formally to substitution of the Cl on the triazine ring with H, followed by protonation, i.e., A₁₉₀ + H⁺. Beam induced halogen substitution reactions have been reported previously (4-6). A free radical mechanism was also used to account for the substitution in references 4-5. An alternative reduction mechanism is presented in reference 6.

Analogous free radical reactions also occur in the analysis of atrazine in other FAB matrices. The reaction scheme to produce m/z 334 is especially important in the FAB analysis of atrazine in a DTT/DTE matrix. This matrix consists of a mixture of dithiothreitol and dithioerythritol both of which have terminal SH groups; the elemental compositions are the same as that of HEDS.

Figure 2 shows a narrow scan over the m/z 216 region of the FAB spectrum (R = 12,000 for 2A-2D and R = 10,000 for 2E & 2F, Kratos MS-50 TA, Kratos FAB source) for several matrices. Both glycerol and 3-nitrobenzyl alcohol (not shown) have background ions which completely overlap the region at which the atrazine protonated molecular ion appears. This point in the window is demarcated with a tic mark. Because thioglycerol contains a S heteroatom, the mass defect of both the matrix and, consequently, the matrix derived background ions is increased. Thus the background ions at m/z 216 in the neat thioglycerol are shifted to lower mass, and appear to the left in the mass window.

Both DTT/DTE and HEDS contain two sulfur atoms. This increases the overall mass defect of these matrix molecules even further than for thioglycerol. This has considerable importance in the trace analysis of atrazine by FAB. The background ions in HEDS are clearly removed from the atrazine region and the peak from the addition of 340 pg of atrazine to the FAB probe tip is also shown in figure 2D. The best detection limits are obtained in the DTT/DTE matrix, and the peak from the addition of 20 pg of atrazine to the probe tip is shown in figure 2F.

Finally, we have also observed matrix analyte free radical chemistry occurring upon Cs⁺ ion bombardment.
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\[
\text{HO-CH}_2\text{-CH}_2\text{-S-CH}_2\text{-CH}_2\text{-OH} \xrightarrow{\text{Ar}} 2\text{-S-CH}_2\text{-CH}_2\text{-OH}
\]

\[H_{77}\]

\[
\text{(CH}_3\text{)}_2\text{CNO}\text{N} \xrightarrow{\text{Ar}} \text{(CH}_3\text{)}_2\text{CNO} + \text{C}^+_{16}
\]

\[A_{160}\]

\[
\text{(CH}_3\text{)}_2\text{CNO} + \text{H}_{77} \xrightarrow{} \text{(CH}_3\text{)}_2\text{CNO}_{167}
\]

\[M_{197}\]

Atrazine

Figure 1. FAB mass spectrum of atrazine in HEDS matrix. Major HEDS matrix ions designated by an H. Protonated products of atrazine and HEDS radical coupling are indicated by a •.

Figure 2. Narrow scans over m/z 216 from: (A) neat glycerol, (B) neat thiglycerol, (C) neat HEDS, (D) HEDS with 340 µg atrazine, mixed on the probe tip, (E) neat DTT/DTE, (F) DTT/DTE with 20 µg atrazine, mixed on the probe tip.

3. K.A. Caldwell and M.L. Gross, presented at the 36th ASMS.
A FAB AND MS/CA/MS STUDY OF ERYTHROMYCIN A AND A SERIES OF RELATED MACROLIDE ANTIBIOTICS

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The mass spectrometric study of thermally labile materials is facilitated by soft ionization techniques such as FAB and SIMS. Both MS/MS and MS/CA/MS provide structural information often unavailable from the simple FAB experiment. We have used these techniques and two tandem sector instruments (Kratos MS-50 TA and VG ZAB-T) to characterize a series of 11 macrolide antibiotics that includes erythromycin A, tylosin, and mycinamicin II. All of the antibiotics studied incorporate from one to five sugar units attached to a 12-, 14- or 16-membered lactone aglycone. At least one of the sugars is an amino sugar. The molecular weights of the glycosides included in this study range from 700 to 1300 u.

The structure of erythromycin A is typical of this class of antibiotics (Fig. 1). Attached to the 14-membered lactone aglycone are two sugar units, desosamine, the amino sugar, and cladinose. The molecular weight of erythromycin A is 733.5 u.

We acquired the low resolution FAB and SIMS mass spectra of the antibiotics as protonated [(M + H)+] and cationized [(M + Li)+, (M + Na)+, and (M + K)+] species to determine the molecular ion. The antibiotics were desorbed from a 3-NBA matrix and 3-NBA saturated with the appropriate salt. High resolution peak matching experiments were performed to confirm the atomic composition of the molecular species. Neither of these techniques give much structural information about the glycosides, however. Characteristic fragmentations are observed in MS/MS spectra, instead. The MS/MS spectra yield information about the structure of the ion of interest, but only if high levels of collisional activation (CA) are employed. Our experiments indicate that the main beam must be reduced by 50-80% with the appropriate pressure of collision gas for some fragment ions to be generated. Metastable ions yield fewer fragment ions than collisionally activated precursor ions.

The MS/CA/MS spectrum of protonated erythromycin A (Fig. 2A) exhibits peaks indicative of bond cleavage accompanied by H rearrangement at the site of the glycosidic oxygen. As a result of this process, the non-amino sugar is lost from the glycoside as a neutral fragment, but the amino sugar is observed as a charged species. Cleavage occurs to either side of the glycosidic oxygen. Loss of H2O is also observed to occur from the (M + H)+ species. Eliminations from the backbone of the aglycone ring are not observed.

Cationized erythromycin A species all produce spectra which are strikingly different from those produced by the protonated species. As is demonstrated in the MS/CA/MS spectrum of (Erythromycin A + K)+ in Fig. 2B, numerous peaks are observed at high mass, but only minor ions appear in the low mass region of the spectrum. Small, neutral molecules are eliminated from the cationized species via cycloreversions of the sugar entities, cleavages to either side of the glycosidic oxygen accompanied by H rearrangement, and H2O loss. Portions of the aglycone ring are also
removed from the cationized species. The same fragmentation patterns are observed for the (M + Lj)+ and (M + Na)+ ions.

![Diagram](image)

Fig. 2: MS/CA/MS Spectra of Erythromycin A

The sharp contrasts observed between the spectra of the (M + H)+ and (M + metal)+ ions of erythromycin A may be the result of differing charge sites. The tertiary amine has high proton affinity, and thus, may hold the proton tightly fixed on the amino sugar. This interpretation is consistent with the observed fragmentations of the (M + H)+ species. The amino sugar appears as a charged entity, but the second sugar is lost as a neutral molecule.

Association of a metal ion with the macrolide occurs on the lactone rather than one of the sugars. Elimination of low molecular weight, neutral molecules from the sugars can occur with the charge remaining on the aglycone portion of the antibiotic. The absence of low mass peaks in the spectra of cationized erythromycin A suggests little metal ion interaction with the sugar portions of the macrolide.

The fragmentation patterns of Erythromycin A are also generally observed for the other macrolides studied here. For all (M + H)+ ions, the amino sugar is observed to be a protonated species and the predominant peak in the MS/CA/MS spectrum. Other fragments include those resulting from loss of H2O and the non-amino sugar. The (M + metal)+ ions all exhibit loss of low mass neutral fragments from the sugar entities and parts of the aglycone.

In conclusion, the molecular ion of macrolide antibiotics can be identified through a combination of low resolution FAB and high resolution peak matching experiments. Structural information about this class of compounds is best obtained with MS/CA/MS techniques. Differences in fragmentation from the (M + H)+ and (M + metal)+ ions occur because the charges are located on different portions of the macrolide molecule.
Molecular Epitope Identification in Protein Antigens by Mass Spectrometric Peptide Mapping Analysis of an Immobilized Immune Complex

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Monoclonal antibodies are powerful tools in biochemistry and medicine. A monoclonal antibody (mab) is specific for a single biomolecule such as a protein, and binds to a specific region on its surface. Current methods for the identification of antigenic sites on proteins depend on panels of peptides or variant proteins as antigenic probes (1). More recent approaches employed partial proteolysis of immune complexes (2,3), revealing a) a general resistance of antibodies towards proteolytic enzymes; b) protection of antigens against proteolytic degradation by a bound antibody; and c) stability of the immune complex after proteolysis of the antigen. But due to major restrictions of the employed techniques, a reliable method of accurate epitope identification is still needed.

Mass spectrometric methods are presented, which are capable of rapidly identifying epitope sequences within a few amino acid residues. Crosslinking of the monoclonal antibody to the insoluble carrier sepharose, allowed the separation of mab-bound molecules from unbound by sedimentation. Two different ways of immune complex formation were developed: 1) the antigen is allowed to bind to the mab, and the resulting immune complex is partially digested by trypsin - this approach is termed "EPITOPE EXCISION"; b) the mab is challenged even by complex mixtures of peptides, resulting in selective binding of only the antigenic peptides - this approach is termed "EPITOPE EXTRACTION". After removal of the supernatant, which can be analyzed separately (non-epitope fraction), the epitope bearing peptides are remaining bound to the immobilized mab and are selectively removed from the mab by the lyotropic agent 4 M MgCl₂ (epitope fraction). Fractions were purified by RP-HPLC and analyzed by ²⁵²Cf-plasma desorption mass spectrometry (PDMS). These methods (4) were applied to the identification of an epitope located on the C-terminus of the complement protein C³a (9 kD), which is recognized by the mab h453 (5).

Fig. 1: PD-Mass spectrometric epitope identification on guinea pig C³a (gpC³a) applying the EPITOPE EXCISION method: 6.4 ng des-Arg⁷⁸-gpC³a was bound to h453 and treated with 0, 5 and 45 ng trypsin respectively (30 min, RT). Epitope
peptides were separated from the mab by treatment with 4 M MgCl₂. Chromatograms of the epitope fractions (right) and the PD-spectrum of the fraction at 23.7 min (left) are shown. The spectrum identifies the C-terminal octapeptide gpC3a(70-77) as epitope peptide and Arg⁶⁹ as tryptic cleavage site (solid arrowhead). In contrast, cleavage in free gpC3a occurred at Arg⁷⁰ (open arrowhead). This change in trypsin specificity can be explained by steric hindrance of trypsin access due to the epitope-bound mab a few amino acid residues downstream.

To further characterize the C-terminal part of the epitope, EPITOPE EXTRACTION analyses were performed using the synthetic peptides hC13 and hC14 and their proteolytic degradation products, which contain the sequence of antigenic human des-Arg⁷⁷-C3a, i.e. ASHLGLA (for sequences see insert).

Fig. 2: EPITOPE EXTRACTION analysis of a) hC13 and hC14; b) tryptic peptide fragments of hC13 and hC14 without and c) with additional digestion by carboxypeptidase B. After binding of the peptide mixtures to immobilized h453, supernatants (non-epitope fraction) and MgCl₂-eluates (epitope fraction) were chromatographed and all eluting peptides analyzed by PDMS, identifying a single antigenic structure, hC14-(1-7), i.e. ASHLGLAGKGKGG (m/z 809). C-terminal elongation of this sequence completely abolished antigenicity. This change in trypsin specificity can be explained by steric hindrance of trypsin access due to the epitope-bound mab a few amino acid residues downstream.

Conclusion An entirely new epitope mapping approach is presented, which allows an accurate description of antigenic structures. The use of an immobilized antibody provides the basis for the antigenic classification of peptides even in complex mixtures. A main feature of the methods presented, is the direct structural identification of molecular species bound to an antibody by mass spectrometric peptide mapping, which prevents experimental artifacts observed with traditional assays (ELISA), e.g. caused by unknown antigenic contaminations. Moreover, the epitope excision method enables the identification of an antigenic structure from a single immune complex and allows access even to the knowledge of steric conditions in the antigen-antibody-protease complex. This method will greatly reduce the number of structures to be tested in fine specificity studies (e.g. using site-directed mutagenesis), and may even be applicable to the complex analyses of assembled topographic sites.

References
COLLISION-INDUCED DECOMPOSITIONS OF PEPTIDE ANIONS CATIONIZED WITH CALCIUM

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We recently presented the collision-induced decomposition (CID) spectra of FAB-desorbed \((M + Ca^{2+} - H)^+\) complexes between peptides and alkaline earth metal ions (1,2). Our results allowed us to deduce the location of the alkaline earth metal ion in the fragmenting complexes from our proposed mechanisms of decomposition (2,3). The most abundant fragment ions arise from complexes in which the metal ion is preferentially bonded to a deprotonated amide group. We also investigated complexes between peptides and alkali metal ions and found that the alkali metal ion binds preferentially to amide carbonyls (1,3-5). The fragmentation patterns of both these complexes change with metal ion size and amino acid sequence, thus providing evidence for intramolecular chelation of the metal ions. Here, we report the study of decompositions of anionic \((M + Ca^{2+} - 3H)^-\) complexes between 45 peptides and calcium, with the intent to provide further evidence of intramolecular chelation between peptides and metal ions in the gas phase.

The CID spectrum of \((M + Ca^{2+} - 3H)^-\) complexes shown in Fig. 1 for VAAF displays characteristic fragmentations observed for complexes containing only aprotic amino acid residues. The important fragment ions are C-terminal \((x_{n-m} + Ca^{2+} - 2H)^-\) and \((y_{n-m} + Ca^{2+} - 2H)^-) ions in which Ca\(^{2+}\) is most likely at the C-terminal carboxylate group. Losses of side chains (see cleavage of Phe in Fig. 1, labelled -Phe) are also abundant.

Spectra of \((M + Ca^{2+} - 3H)^-\) complexes of VIHN, AEKAA, and YGGFL (Fig. 2) show characteristic fragmentations for peptide complexes that contain protic amino acids. Fragmentations yield \(x_{n-m}\) and \(y_{n-m}\) ions and losses of amino acid side chains. In addition, however, decompositions also produce N-terminal \((c_{n-m} + Ca^{2+} - 2H)^+\) fragment ions. The \(c_{n-m}\) ions are observed in spectra of peptides that contain at least one His (Fig. 2A), Glu (Fig. 2B), Tyr (Fig. 2C), or Asp. Losses of amino acid side chains dominate spectra of peptide complexes that contain other protic side chains such as Arg, Ser, Thr, and Asn (see Fig. 2A, labelled -Asn).

The mechanism for formation of \((c_{n-m} + Ca^{2+} - 2H)^+\) ions appears to be the same as that proposed earlier for \((c_{n-m} + Ca^{2+})^+\) ions (2,3). The \(c_{n-m}\) ions arise from cleavages through OCN(H)-C(H)R bonds, require transfer of an \(\alpha\)-hydrogen from an R group on the departing neutral to the amide N of the fragment ion, and are inhibited by proline in the \(n-m+1\) position. Calcium is located at a deprotonated amide towards the N-terminus and distant from the reaction site. Formation of \((c_{n-m} + Ca^{2+} - 2H)^-\) ions also requires at least one more amide bond and a protic side chain that are deprotonated and involved in binding calcium. The necessity for multiple binding sites for calcium in \((c_{n-m} + Ca^{2+} - 2H)^-\) ions provides support for intramolecular chelate complexes.
Fig. 2 CID spectra of (M + Ca$^{2+}$ - 3H)$^+$ complexes of VIHN (A), AEKAA (B), and YGGGL (C).

References
OPEN CELL GEOMETRIES FOR FOURIER TRANSFORM MASS SPECTROMETRY

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A variety of cell designs have been employed for Fourier transform mass spectrometry (FTMS) with the most common being of cubic, rectangular, and cylindrical geometry. All implementations of these designs to date have employed trap electrodes oriented perpendicular to the excite and detect electrodes. Such orientation results in a closed cell exhibiting several disadvantages for the FTMS experiment including restricted access to the cell interior and reduced gas conductance through the cell. Perhaps the most significant disadvantage of such designs is the inhomogeneous electric fields which result from the termination of field lines on adjacent electrodes. This effect causes significant field curvature and thus introduces an undesirable radial component to the trapping field and axial component to the excitation field. The radial trapping field component induces magnetron motion and induces a negative shift in the cyclotron frequency, and the axial excitation field component results in axial ejection of ions during excitation. In this work we present an alternative “open” cell geometry achieved by employing trap electrodes which are a linear extension of the geometry defined by the excite and detect electrodes. In contrast to closed cells, this open cell geometry offers several advantages including easy access to the cell interior, improved gas conductance through the cell, and significantly reduced electric field inhomogeneities. The absence of physical barriers along the cell axis simplifies the introduction of externally generated charged particles or laser beams, and the improved conductance through the cell region shortens pressure transients associated with laser desorption and pulsed gas introduction. The efficient injection of externally generated ions is also facilitated in that the possibility of trapping surface interactions are eliminated, as well as interactions with magnetic field inhomogeneities associated with paramagnetic trap plate materials. Improved electric field homogeneity results in smaller trap potential induced cyclotron frequency shifts and reduced axial ejection associated with excitation fields.

An example of this open cell design approach is presented in Figure 1 for cells of both square and circular cross section. This design results in a cell with three tandem colinear sections consisting of two trapping sections and one excite/detect section. The length of the trap extensions will determine the maximum centerline trapping potential achieved for a given applied potential, with length/width (aspect) ratios as small as 1/15 yielding effective potential wells. However a more effective ratio of 1/1 results in a trap potential maxima that is 92% of the applied potential and located essentially at the center of the extension. The length of the trapping potential well is then easily determined as the distance between trap extension centers. If the aspect ratio of the excite and detect electrode assembly is also 1/1, and the length of the cell is taken as the length of the potential well, then the overall aspect ratio of the cell is 2:1. Aspect ratios of greater than 1/1 are typically referred to as elongated cells. A field map showing the trapping field isopotential contours for such a cell is presented in Figure 2 along with a corresponding map for the closed elongated cell of the same aspect ratio. That this open cell design is capable of generating suitable trapping potential wells is demonstrated in Figure 3 where the centerline trapping potential for both the open and closed elongated cells are compared. It should be noted that although the closed cell exhibits a slightly deeper effective well, the open cell geometry results in significantly smaller peak radial electric fields.

Isopotential contours for the excitation fields of both the open and closed elongated cells are presented in Figure 4. While the closed cell offers a more homogeneous radial excitation field as averaged over the length of the cell, the open cell exhibits axial field gradients that are approximately 1/2 that of the closed cell. This results in reduced axial ejection during excitation, as is demonstrated with perfluorotributyl amine (PFTBA) spectra presented in Figure 5. Further modification of the open cell design can eliminate ejection entirely. If the trapping extensions are cut into quadrants corresponding to the excite and detect plates, the excitation waveform can be superimposed on the trap potentials applied to those trap extension segments which are colinear with the excite plates. As demonstrated in Figure 6, the resulting excitation field is homogeneous over the entire trapping potential well, thus significantly improving excitation performance of the open cell as compared to closed cell designs.

Another advantage of the open cell design is the increased experimental versatility which may be realized by extending the open-colinear section concept to more than three sections. For example, with the appropriate application of trapping and excitation potentials to different sections, a five section cell could be configured to perform as a single cell of variable aspect ratio, or as a tandem cell. Such a configuration is shown schematically in Figure 7.
Open Geometry Trapped Ion Cells
Trapping Electrodes Collinear with Magnetic Field

Examples

Isopotential Field Maps for Trapping Electrodes = 1 V

Closed Elongated Cell

Open Elongated Cell

Fig. 1

Elongated Cell Centerline Trap Potential

Isopotential Field Maps for Excitation Electrodes

Closed elongated cell

Open elongated cell

Fig. 2

Axial Ejection in El Spectra of PFTBA

Closed Cell 1V Trap 98V Excite

Fig. 3

Open Cell 1V Trap 98V Excite

Fig. 5

Isopotential Field Maps for Excitation on Excite and Trapping Electrodes

Fig. 4

Open Cell Versatility: The Five Section Open Cell

Fig. 7
Resonant Excitation for GC/MS/MS in the Quadrupole Ion Trap via Frequency Assignment Pre-Scans and Broadband Excitation

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Two of the quadrupole ion trap's most notable features are ultra-high sensitivity as a gas chromatograph/mass spectrometer (GC/MS), and versatility as an efficient tandem mass spectrometer (MS/MS). GC/MS/MS would seem to be the obvious next step, and although GC/MS/MS has been demonstrated with the ion trap, several issues need to be resolved before GC/MS/MS becomes a routine mode of analysis. The combination of GC and MS/MS in the ion trap is complicated by the interdependence between operating parameters that affect MS/MS and the ion population in the trap at any given time. In particular, as the number of the ions within the trap change during the elution of a chromatographic peak, the proper setting of the resonant excitation frequency shifts. Figure 1 illustrates this shift in a plot of the parent ion intensity for 134* of n-butylbenzene against resonant excitation frequency for three different ion densities. As the number of ions in the trap increases, the excitation band shifts to lower resonant excitation frequencies. This often results in the loss of daughter ion production over part or all of the GC peak.

We have developed two techniques that correct for the shift in resonant frequency in real-time. The first technique applies a frequency-assignment pre-scan that automatically determines the resonant ion frequency for a subsequent MS/MS scan. The second approach eliminates the need for exact frequency assignment by exciting a range of frequencies simultaneously with a broadband excitation signal.

The frequency-assignment pre-scan (Figure 2) rapidly measures the secular frequency of an ion's motion with a frequency scan acquisition. Data obtained by scanning the resonant excitation frequency across a 10 KHz (1.7 amu) range (Figure 3). As the synthesizer passes over the an ion's resonant frequency, power is absorbed and the ion is ejected from the trap to the detector. Appropriate resonant excitation frequencies are obtained by calculating the average ejection frequency for increasing and decreasing frequency scan acquisitions; averaging the scans corrects for additional frequency perturbations that are caused by the amplitude of the frequency scan. Less than 100 μs is required for accurate frequency assignment, which we believe will be fast enough to perform MS/MS over a chromatographic peak. In practice, the frequency-assignment pre-scan takes 1-2 additional seconds for communication between the data system and the instruments embedded microprocessor; the elimination of these delays is possible with additional software modifications.

Broadband excitation applies an excitation signal that has a bandwidth of approximately 10 KHz (1.7 amu at q=0.3) during the CAD stage of an MS/MS experiment. Thus, the excitation of the parent ion is independent of slight shifts in the ions secular frequency of motion. In addition, the broadband excitation scan function is faster and less complex than the frequency-assignment pre-scan. Broadband excitation is found to be an effective method for resonant excitation during the GC/MS/MS experiment and is a significant
step towards routine GC/MS/MS on the quadrupole ion trap. Figure 4 shows the GC/MS/MS results for n-butylibenzene; note that daughter ions 91' and 92' are observed over the entire chromatographic peak. One potential disadvantage of the broadband excitation technique is that it places strict requirements on the mass selection step; if unit mass-selection is not achieved and multiple m/z ions remain following isolation, broadband excitation may produce an ambiguous daughter spectrum containing daughter ions from multiple parent ions.

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\( \text{Shlfts in the Resonant Excitation} \)
\( \text{Frequency Caused by the Density of} \)
\( \text{Ions in the Ion Trap} \)

- **- 1 -**

Frequency-Assignment Pre-Scan and MS/MS Scan

- **- 2 -**

Frequency-Scan Acquisitions — User Interface

- **- 3 -**

GC/MS/MS — Broadband Excitation

(800 pg n-butylibenzene; Daughters of 134')
Determining Effective Ion Energies in a Quadrupole Ion Trap Mass Spectrometer

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We are conducting a series of experiments which should lead to the determination of the energy of the ions stored in a quadrupole ion trap mass spectrometer (QITMS). As the QITMS sees increased use as a routine analytical mass spectrometer, it is becoming more important to understand - if not ultimately to control - the energy deposition processes occurring in the ion trap. To this end, we have utilized a series of chemical “thermometer” reactions in an effort to characterize the energies of the ions in the QITMS.

The first two chemical “thermometers” studied were the ion-molecule reactions,

\[
\text{Ar}^+ + \text{N}_2 \rightarrow \text{Ar} + \text{N}_2^+ \tag{1}
\]

and

\[
\text{O}_2^+ + \text{CH}_4 \rightarrow \text{CH}_3\text{O}_2^+ + \text{H} \tag{2}
\]

Flow-DRIFT experiments have shown that the rate coefficients, \(k\), for these reactions vary with centre-of-mass kinetic energy, \(K_E\).[1,2] Thus, determination of \(k\) for these “kinetic thermometers” on the QITMS leads to a value for the \(K_E\) of the reacting ion-molecule pair. An effective temperature, \(T(K)\), can be derived by transforming to \(K_E\) and applying the relation \(K_E = 3/2kT\) (assuming a Maxwell-Boltzmann distribution for the ion energies).

The second type of chemical “thermometer” utilized is based on determination of the equilibrium constant, \(K_{eq}\), for a proton transfer reaction. Given knowledge of either the \(\Delta G\) or \(\Delta H\) for the reaction, an effective temperature, \(T(K)\), can be obtained from these “thermodynamic thermometers” via the relation, \(\Delta G = \Delta H - T\Delta S = -RT\ln K_{eq}\) assuming \(\Delta S = 0\) or estimating it as appropriate. The two proton transfer equilibria studied were those between protonated toluene and ethyl benzene and between n-diethyl amine and n-dipropyl amine,

\[
\text{C}_7\text{H}_9^+ + \text{C}_8\text{H}_{10} \rightarrow \text{C}_8\text{H}_{11}^+ + \text{C}_7\text{H}_8 \tag{3}
\]

and

\[
[(\text{n-Et})_2\text{NH}]^+ + [(\text{n-Pr})_2\text{NH}]^- \rightarrow (\text{n-Et})_2\text{NH} + [(\text{n-Pr})_2\text{NH}]^+ \tag{4}
\]

The effective temperatures obtained from the four “thermometers”, both without and with He buffer gas (added to a total pressure of \(1\times10^{-4}\) torr) in the QITMS are given in Table 1. The result for the \(\text{Ar}^+ / \text{N}_2\) “thermometer” without He buffer is the average obtained over an \(\text{Ar} / \text{N}_2\) pressure range of \(1\times10^{-6}\) to \(1\times10^{-3}\) torr (\(\text{Ar} / \text{N}_2\) pressure ratio varied from 10:1 to 1:10). Both the \(\text{O}_2^+ / \text{CH}_4\) and toluene/ethyl benzene temperatures without He buffer were obtained at a pressure of \(5\times10^{-6}\) torr (50:50 neutral ratio). The result for the amine “thermometer” without He is the average value obtained over the pressure range \(7\times10^{-7}\) to \(5\times10^{-5}\) torr (2:1 n-diethyl:n-dipropyl amine ratio).

Results to date show that the kinetic “thermometers” give ion temperatures a factor of 10 higher than those obtained with the thermodynamic “thermometers”. Without He buffer gas, a temperature of approximately 3500K is given by both the \(\text{Ar}^+ / \text{N}_2\) and \(\text{O}_2^+ / \text{CH}_4\) “thermometers”, while the toluene/ethyl benzene and the n-diethyl/n-dipropyl amine “thermometers” result in temperatures on the order of 300K. In all cases somewhat of a cooling effect is seen upon addition of the He buffer gas.

The reason for the ten-fold difference in temperature between the kinetic and thermodynamic “thermometers” is as yet unclear; we suspect, however, that it may lie in the transformation from \(k\) to \(T(K)\). It is interesting to note that if the temperature-variable SIFT DRIFT data available for the \(\text{O}_2^+ / \text{CH}_4\) “thermometer” (providing a direct measure of \(T(K)\) from \(k\) [3]) are utilized instead of the flow-DRIFT results, temperatures on the order of 560K are obtained. These are not only in better agreement with the thermodynamic “thermometers”, but are in good agreement with the 600-700K temperature range reported previously for the ions in a QITMS [4] utilizing the temperature-variable SIFT DRIFT \(\text{O}_2^+ / \text{CH}_4\) “thermometer”.

While we have yet to determine the absolute values for the temperatures of the ions stored in a QITMS, trends in ion temperatures obtained with the \(\text{Ar}^+ / \text{N}_2\) “thermometer” as a function of the \(q\) of the \(\text{Ar}^+\) ion (Figure 1) indicate that the “thermometer” is indeed probing ion energy.
Table 1. **Effective Ion Temperatures**

| Kinetic | **no He buffer** | | | **He buffer** | | |
|--------|------------------|------------------|------------------|------------------|------------------|
| | $K.E_{cm}$ (eV) | $K.E_{ion}$ (eV) | $T$ (K) | $K.E_{cm}$ (eV) | $K.E_{ion}$ (eV) | $T$ (K) |
| Ar$^+$/N$_2$ | 0.19±0.07 | 0.40 | 3100±700 | 0.14±0.01 | 0.26 | 2000±300 |
| O$_2^+$/CH$_4$ | 0.20±0.04 | 0.50 | 3900±200 | 0.17±0.06 | 0.43 | 3550±650 |

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<td>toluene/ethyl benzene</td>
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<td>diethyl/dipropyl amine</td>
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<td>305±20</td>
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Figure 1. Effects of He buffer and RF field (Mathieu q parameter for Ar$^+$) on the ion temperatures as measured by the Ar$^+$/N$_2$ kinetic "thermometer".

References:
The great analytical potential of electrospray ionization for analysis of both light and heavy molecules has led us to retrofit an existing HP 5988A (Hewlett Packard, Palo Alto, CA) quadrupole mass spectrometer with a commercially available electrospray source and a compatible high energy dynode (HED) detector. Our evaluation of this instrumental configuration included a qualitative comparison of electrospray spectra with those in the literature and assessments of sensitivity and mass accuracy. We determined that this retrofitted configuration using multiple suppliers can provide excellent analytical capabilities for electrospray mass spectrometry. The HED detector (Phrasor Scientific, Duarte, CA) gave an optimal signal at a dynode voltage of -7.5 kV which represented an increase of two orders of magnitude over a conventional conversion dynode electron multiplier. The high mass sensitivity was improved with the HED as well. The electrospray source (Vestec Corporation, Houston, TX) replaced the existing Hewlett Packard EI/CI source with no modification necessary.

A glutathione and a glucuronide were used to test the sensitivity of this configuration. Methanol content of the infusion solvent had little effect on sensitivity of the molecular ion detected between 1.0 and 0.01 nmol. A lower limit of 780 fmol per scan was detected for both the glutathione and the glucuronide. This represents a concentration of 0.01 nmol of solution undergoing continuous infusion in the syringe. The mass accuracy for all samples tested is within a 0.02 - 0.005 percent error range.

Manipulation of electrospray parameters can provide additional spectral information. On increasing the block/repeller voltage, fragmentation was found to occur which was similar to that of high energy collision induced dissociation. Proteins of molecular weight exceeding the mass range of the quadrupole (2000 D) was were found to yield singly and multiply charged fragment ions (Figure 1).

High molecular weight compounds could be analyzed with the addition of 20 - 30 percent acetic acid. An example is alcohol dehydrogenase from yeast of mass ~ 74 kDa which has up to 70 positive charges (Figure 2). Analysis of glycoproteins and protein mixtures were also found to be feasible. Negative ion electrospray of compounds such as nucleotides resulted in multiply charged negative ion distributions. Analysis of real samples such as the P-17 fragment of the gag protein from the HIV-I virus gave comparable results to test compounds.
Figure 1: ES/MS of melittin at different repeller voltages showing the induction of fragmentation at a higher voltage.

Melittin (R = 20 V)

Melittin (R = 50 V)

Figure 2: ES/MS of alcohol dehydrogenase from yeast.

Alcohol Dehydrogenase

MW = 73534.5 D
HOAc:H2O:MeOH
3:3:4

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Alzheimer's disease (AD) is neuropathologically characterized by deposits of amyloid, the senile plaque in cerebral blood vessels and the neuropil. The senile plaque is a fibrillar extracellular deposit primarily composed of a ca. 4-kilodalton peptide, β/A4, derived from the amyloid precursor protein (APP) (Fig. 1.A). The 42-43 amino acid β/A4 peptide includes 14 amino acids of the transmembrane domain and 28 amino acids of the adjacent extracellular domain of APP. However, the biochemical events responsible for the generation of β/A4 from APP are not clear. Studies performed in cultured cell have documented that APPs have a short intracellular half-life and are posttranslationally modified both by N- and O-linked carbohydrate and by tyrosine sulfation. Full-length molecules are detected on the cell surface, and proteins lacking the transmembrane and cytoplasmic domains are secreted into the medium. Similar events occur in vivo.

Recently, we identified a domain of APP that was necessary and sufficient for this constitutive cleavage event. We provided compelling evidence that this constitutive cleavage occurs in the extracellular domain, within the β/A4 peptide (Fig. 1.B). Subsequently, Esch et al. confirmed these molecular biological studies by chemically identifying unique carboxyl- and amino-terminal residues of the secreted and membrane-retained fragments respectively. However, the precise cleavage site was indeterminate because a lysine residue (Fig. 1.B), which normally resides between the identified termini of secreted and membrane-retained fragments, was not detected in this analysis.

To define the precise APP cleavage site that leads to secretion, we have now generated a Chinese hamster ovary (CHO) cell line that overexpresses the human APP-770. The soluble APP-770 (SAPP-770) was purified from the conditioned medium of these cells by Mono Q HR5/5 anion exchange FPLC and analyzed by SDS/PAGE with silver staining and immunoblotting. The purified SAPP-770 was chemically digested with cyanogen bromide (CNBr). Peptides were separated by Vydac C-18 reverse-phase HPLC (Fig. 2A). The peptide contains carboxyl-terminal residues of SAPP-770 were
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Identified by plasma desorption mass spectrometry (PDMS) (Fig. 2B and 2C) and verified by Edman degradation (Fig. 3) and in situ carboxypeptidase Y digestion on PDMS sample foil (Fig. 4).

A portion of the reverse-phase HPLC profile of peptides generated by CNBr digestion of SAPP-770 is shown in Figure 2A. This region of the chromatogram was selected for analysis because a synthetic 16 amino acid peptide, DAEFRHDSQYEVHHQK, had retention time of 66 minutes under identical chromatographic conditions. To confirm that an authentic CNBr generated C-terminal peptide of SAPP-770 fractionated in this region of the chromatogram, we tested each of the fractions by PDMS. This initial PDMS survey revealed that fractions 66 and 67 of the chromatogram (Fig. 2A) contained peptides with molecular weights appropriate for a CNBr generated C-terminal peptide of SAPP-770. Figure 2B depicts the PDMS analysis of fraction 66 showing a strong peak of m/z 1955.6 and a weaker peak of m/z 1827.1. Similarly, Figure 2C depicts PDMS analysis of fraction 67 showing peaks identical to fraction 66 but with reversed intensity. These two [M+H]+ ions of 1955.6 and 1826.9 are correspond to the molecular weights of CNBr digest of SAPP-770 with the sequence DAEFRHDSQYEVHHQ (calculated m/z = 1955.6) and DAEFRHDSGYEVHHQ (calculated m/z = 1826.9), respectively. Note that the predicted difference in hydrophobicity of these two peptides is reflected by the respective retention on the reverse-phase column.

![Figure 3 Edman degradation of C-terminal CNBr derived peptides of SAPP-770. Amino acids of the highest yields are shown at each sequence cycle](image)

The Edman degradation analysis (Fig. 3) revealed that fractions 66 and 67 do contain peptides with amino acid sequences that matched the APP sequence. The in situ carboxypeptidase Y digestion on PDMS sample foil (Fig. 4) further confirmed the C-terminal amino acid sequence of the ion m/z 1955.6 is ...QK by the mass difference of between the original and first digest ions, and between first and second digest ions.

These data provide the evident that the primary site for the membrane associated cleavage of APP-770 is immediately N-terminal to leucine 688, thereby generating a secreted molecule (i.e., SAPP-770) with a C-terminal lysine residue. A postcleavage event involving exopeptidase removal of lysing 687 would then give rise to secreted molecules with a C-terminal glutamine residue (as predominate in fraction 67).

References
A METHOD FOR BACKGROUND REDUCTION IN A SUPERSONIC JET/MULTIPHOTON IONIZATION REFLECTRON TIME-OF-FLIGHT MASS SPECTROMETER

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Multiphoton ionization mass spectrometry (MPIMS) combined with supersonic jet spectroscopy (SJS) is a powerful technique for trace analysis and for the determination of chemical structures. In the past few years, in an effort to extend both the MPIMS and the SJS method for the study of thermally labile biochemicals, pulsed laser desorption (LD)[1,2] and, more recently, fast atom bombardment (FAB)[3] have been used to vaporize these molecules. In the LD or FAB experiments, a pulsed laser (e.g., CO2 laser) or a FAB gun is used to desorb molecules from a sample substrate placed close to the nozzle orifice. The resulting neutral molecules are then entrained into a pulsed supersonic jet which prevents them from thermal decomposition through collisional cooling and carries them into a time-of-flight mass spectrometer (TOFMS) where MPI takes place. Both LD and FAB/SJ-MPI mass spectrometry methods have been applied for the detection of a variety of biochemicals[1-3].

As in most other mass spectrometry techniques, high-intensity background can cause some problems in SJ-MPIMS. This is particularly true for FAB and LD/SJ-MPIMS. In these techniques, the nonvolatile molecules are first vaporized and then carried into the ionization region of the TOFMS. During this process, samples may condense on the wall of the vacuum chamber and the electric lens assembly of the TOFMS. These molecules and their decomposition products will eventually become the background for the analysis of other samples or the same sample in the next run. This cross-contamination can become quite severe in light of the fact that the turn-around time of the system is usually very short (i.e., less than 5 minutes) and a large number of samples can be analyzed in a given time period. Due to the possible overlapping of the molecular ion and fragments with the background signals, the sensitivity for the detection of molecules of interest is often reduced, and also the structural information obtained from the mass fragmentation pattern suffers.

In this report, we describe a method for background reduction in FAB and LD/SJ-MPIMS. This method involves (1) the use of an ion deflector in a supersonic jet/reflectron TOFMS, and (2) the optimization of the time delay between the sample pulse and the ionization laser pulse, to selectively detect the sample ions from background interference. Note that the ion deflector placed above the acceleration plate is commonly used in supersonic jet TOFMS for controlling the trajectory of ions from the ionization region to the detector. Now if the sample in the jet expends to the ionization region at a velocity perpendicular to the acceleration field or ion flight axis, the ions generated from the sample(sampling-ion packet) will have different linear velocity from the ions originated from the background molecules(background-ion packet). Thus they will travel to the detector with different trajectories. If there is a sufficient spatial separation between these two ion packets when they arrive at the detector, then, by selection of a proper ion deflector voltage, the sample-ion packet can be selectively detected by the detector. We show here that in our linear-type TOFMS, it is difficult to use the ion deflector to reduce the background in FAB and LD/SJ-MPI experiments. However, we demonstrate that the background can be significantly reduced by using the ion deflector in a reflectron TOFMS.

In this study, the linear velocity difference between the sample molecules in the jet and the background molecules is first examined. Then a computer simulation is performed to study the ion trajectories of the ions entrained into the TOFMS with various initial linear velocities or initial kinetic energies. The simulation indicates that, because the ion flight distance is doubled in the reflectron TOFMS, the spatial separation of the sample-ion packet and the background-ion packet can also be doubled. Moreover, with the reflectron TOFMS, the initial kinetic energy differences among the sample ions can be partially compensated for by applying proper voltages to the reflector to focus the sample ions, which results in a narrower energy distribution within the sample-ion packet. The reflector will also compensate, to some extent, for the energy differences among the background ions. The combination of the longer flight distance and less diffused sample-ion packet enhances the spatial separation between the two ion packets. Thus it becomes possible to selectively detect the sample ions.
The application of this background reduction method for FAB and LD/SJ-MPIMS is illustrated in Fig. 1. Figure 1A is the mass spectrum of aniline (m/z=93) and background obtained by using laser ionization at 266 nm with an ion deflector voltage of 70 V. Figure 1B shows the mass spectrum of 9,10-dimethylanthracene obtained by using laser desorption for sample volatilization with MPI at 266 nm. The deflector voltage is also set at 70 V. Note that the molecular ion peak (m/z=206) is superimposed on the background. This problem makes the quantification difficult. The mass spectra shown in Fig. 1C and D are obtained under the same conditions as in Fig. 1A and B, respectively, except that the ion deflector voltage is set at 0 V. As Fig. 1C and D show, the background is now significantly reduced, and the small fragment ion (m/z=191) from the loss of CH$_3$ from the molecular ion peak can be clearly identified. This example represents a typical experiment for background reduction performed in FAB and LD/SJ-MPI studies of nonvolatile chemicals. This method has now been routinely used in our lab.

References


![Figure 1. MPI mass spectra of (A) aniline with background, (B) aniline and 9,10-dimethylanthracene with background, (C) aniline, and (D) aniline and 9,10-dimethylanthracene. Aniline is introduced into the jet directly with the expansion gas; 9,10-dimethylanthracene is introduced into the jet expansion by using laser desorption. The ion deflector voltage is 70 V for A and B, 0 V for C and D.](image-url)
ORGANIC SECONDARY ION YIELD MEASUREMENTS WITH A PULSED, TIME-OF-FLIGHT MASS SPECTROMETER

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We are investigating the processes of desorption and ionization from liquid matrices by bombarding nucleosides, nucleotides, and a few other organic molecules with pulses of energetic (keV) metal ions in a time-of-flight (TOF) mass spectrometer. The instrument has been described in detail elsewhere [1,2]. Briefly, positive primary ions of In, Ga, Bi, Au, or Si are generated and accelerated to kinetic energies of 10-50 keV in a focusing liquid metal ion (LMI) column. The LMI column is coupled to the mass spectrometer's ion source through a Wien filter that separates the various ionic species in the primary beam according to their isotopic identities and charge states. We have detected down to 50 femtomoles of gramicidin-S (1141.4 Da) and 15 picomoles of various nucleotides (Figure 1) in the positive ion mode with this instrument. These detection limits are about 50 times better than we observe using conventional FAB techniques.

We are studying the physical processes of ion ejection by measuring the relative secondary ion yields of nucleosides and nucleotides from glycerol matrices bombarded by the different ionic species at our disposal. All samples were prepared in water solution (0.025-0.25 μg/μL); 0.2 μL of sample solution were mixed with 5 μL of glycerol before being loaded into the mass spectrometer.

Typical secondary ion yield data from our measurements are shown in Figures 2-6. Yield in these figures is defined as the total number of secondary ions detected divided by the total number of impinging primary ions. Specifically, Figure 2 and 3 show the secondary ion yields obtained respectively by bombarding 40 ng samples of various deoxynucleosides and 50 ng samples of different deoxynucleotides with In and Bi ions. Figure 4 shows the secondary ion yield of deoxyguanosine monophosphate (dGMP) as a function of the energy of the primary ions. Figure 5 and 6 show the secondary ion yields (normalized with respect to the yield from In⁺ bombardment) respectively of deoxyadenosine (dA) and dGMP due to bombardment with In, Au, Bi, and some of their cluster ions.

The data presented in Figures 5 and 6 show that the relative ion production of protonated dA and dGMP secondary ions under polyatomic cluster ion bombardment is increased by up to 16 times over monatomic ion bombardment. Similar results for an organic analyte were obtained by Salehpour et al. when they bombarded valine on a solid substrate with 800 keV to 3.7 MeV polyatomic C₇H₃⁺ (n=1,3,4,7; m=0,3,9,15) ions [3]. The data generated by bombardment with monoatomic In and Bi ions suggest that the secondary ion yield is proportional to the momentum of the primary ion. The very small change in secondary ion yields with energy probably reflects the facts that we are observing a nuclear sputtering phenomenon and that the energy range of our measurements (12-18 keV) was quite narrow.

Acknowledgement
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References
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Figure 1

Deoxyadenosine monophosphate $5\mu$g in glycerol

Intensity (pmoles)

Secondary Ion Yield of Nucleotide

$5\mu$g in glycerol, Positive Ion Mode

Bombarded with In, Br, Bi

Figure 2

Secondary Ion Yield of Nucleotide

$5\mu$g in glycerol, Positive Ion Mode

Bombarded with 13 kV In, Br, Bi

Figure 3

Secondary Ion Yield of Nucleotide

$5\mu$g in glycerol, Positive Ion Mode

Bombarded with In, Br, Bi, Cl cluster ion and Br cluster ion

Figure 4

Secondary Ion Yield of Nucleotide

$5\mu$g in glycerol, Positive Ion Mode

Bombarded with In, Br, Bi, Cl cluster ion and Br cluster ion

Figure 5

Secondary Ion Yield of Nucleotide

$5\mu$g in glycerol, Positive Ion Mode

Bombarded with In, Br, Bi, Cl cluster ion and Br cluster ion

Figure 6

Secondary Ion Yield of Nucleotide

$5\mu$g in glycerol, Positive Ion Mode

Bombarded with In, Br, Bi, Cl cluster ion and Br cluster ion
THEORETICAL ANALYSIS OF THE EFFECT OF ELECTRIC FIELD INHOMOGENEITIES IN THE AREA OF WIRE MESH GRIDS ON RESOLUTION IN THE TIME-OF-FLIGHT MASS SPECTROMETER

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Laser ionization and laser desorption techniques are important ionization methods for the analysis of both synthetic and biological polymers. Recent work with matrix-assisted ultraviolet laser desorption has demonstrated its ability to generate intact molecular ion of large (upwards of 250,000 daltons) proteins and peptides. The time-of-flight mass spectrometer (TOFMS) meets the unique requirements of extended mass range and the ability to work with these pulsed ionization techniques, but typically suffers from relatively poor mass resolution. Higher mass resolution would provide unambiguous information about adduct formation and small subunit variations for these soft ionization techniques, and improved data on fragmentation and molecular structure when combined with harder ionization methods.

Most TOFMS employ ionization/acceleration regions defined by fine mesh metal grids. The resulting position dependent potential gradients degrade mass resolution by increasing the ion time of flight distribution. Results of mathematical modeling of the effects of changing the electric field difference across the grid and of grid mesh size are presented for several different mass ions. The importance of the effect is measured by calculation of the time-of-flight differences that are a result of ions passing through the grid.

The electric potential array calculated by SIMION2 was used to calculate the ion time of flight spread as a result of passing through wire mesh grids. Figure 1 shows the distribution of flight times for several ions of the same mass formed at the same time with the same initial kinetic energy at several positions along the grid. The time spread is a result of the position dependent electric fields, i.e. the curved equipotential lines shown in figure 2.

The time spread that results is mass dependant. Larger mass ions spend more time in different electric fields and thus spread further as they pass through the grid (figure 3). The initial velocity at the grid has a similar effect. The slower ions spend more time in different electric fields and thus have larger time spreads.
Another factor affecting the time spread is the electric field difference across the grid. The larger the electric field difference, the more the electric field punches through the grid. This gives larger electric field difference for each position along the grid and thus a larger flight time spread.

The time spread is also affected by the grid mesh size used. The larger mesh sizes have more wires per inch which results in more constant electric fields and smaller flight time spreads (figure 4).

The time variation due to the grid effects is compared to the flight time distributions created by the length of the initial ionization pulse, the initial velocity distribution, and the initial spatial distribution of ionization. The results suggest that the grid effects can be reduced while maintaining the high accelerations needed for enhanced detection of large ions. Preliminary results from a linear TOFMS designed to reduce these grid effects will also be presented.

2. D.A. Dahl and J.E. Delmore, SIMION PC/PS2, version 4.0, Idaho National Engineering Laboratory, EG&G Idaho Inc., P.O. Box 1625, Idaho Falls, ID 83415.
A TIME-OF-FLIGHT MASS SPECTROMETER FOR PHOTODISSOCIATION STUDIES OF PEPTIDES AND PROTEINS

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Matrix assisted laser desorption is a powerful analytical method for making accurate molecular weight determinations of proteins as large as 300 kDa from sub-picomolar quantities of sample (1,2). Protein ions formed during the desorption process are primarily singly charged molecular species exhibiting little or no fragmentation. As a result, few deductions can be made concerning the structure of the protein molecule.

Structurally significant fragments of peptide molecular ions have been obtained through the use of laser photodissociation (3). Irradiation with laser light provides a convenient means of depositing discrete amounts of energy into a molecular ion. The amount of energy deposited is dependent on the laser wavelength, and not on the mass of the parent ion, in contrast to collisionally activated dissociation.

In order to investigate photodissociation of protein ions produced by matrix assisted laser desorption, we have designed and constructed the reflectron time-of-flight mass spectrometer shown in figure 1. Ions are desorbed from a stainless steel sample probe which fits flush with the repeller plate of a two stage acceleration region. After passing through an Einzel lens and beam steering optics, the ions are focused into a single stage, 59 cm reflectron consisting of 46 rings. The reflected ions then strike an electron multiplier tube for detection. The total flight distance is approximately 3 m. Fused silica windows located in the source and reflection regions are used to introduce ultraviolet light for photodissociation experiments.

In addition to providing increased resolution for molecular weight determinations, the reflectron offers some advantages for photodissociation experiments as well (4). At the acceleration voltages typically employed, 5-15 kV, ion lifetimes in the turning region of the reflectron are on the order of several microseconds. Comparing this value to a value of a few hundred nanoseconds for a 1 cm flight path just outside the initial acceleration region, one can see that timing concerns for the triggering of the photodissociation laser are less critical for photodissociation occurring in the reflectron. Another advantage over previous photodissociation experiments in a time-of-flight instrument (5) is the ability to selectively fragment a particular component of a mixture of compounds having comparable masses. At the turning point in the reflectron, ions having only slightly different masses will be sufficiently separated in time. There will then be little chance of the photodissociation beam overlapping any ion packet other than that having the targeted mass. This feature will be particularly useful for analyzing protein mixtures.

An important concern to be addressed by these experiments is whether large protein ions will photodissociate on the microsecond time scale of the experiment. Beginning at the turning point, the flight time for passage through the reflectron is 50-100 μs. Daughter ions formed away from the turning point or outside the mirror region will not be detected at their correct mass. Statistical bond dissociation may occur too slowly to be observed for truly large ions. Increasing the energy deposited into a parent ion by multiphoton photodissociation may be useful for increasing the rate of the photodissociation process, and will be investigated in future studies.

REFERENCES:

Figure 1. Schematic of a time-of-flight mass spectrometer for photodissociation studies. The inset shows the source components and ion optics.

Figure 2. SIMION calculations of the trajectories of a m/z 5000 parent ion, and m/z 4000, 3000, 2000, and 1000 daughter ions from dissociations occurring at the turning point of the parent ion trajectory in the mirror region. The vertical lines represent the cross sectional view of the reflectron mirror. The parent ion enters along the top trajectory to the left, and exits along the bottom trajectory to the left. Lighter daughter ions are deflected closer to the central axis of the mirror, with m/z 1000 having the trajectory closest to the center.
Design and Performance of a New Linear TOFMS for Matrix Assisted Laser Desorption/Ionization

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Recently the success of matrix assisted laser desorption/ionization (LD/I) for introduction of high mass ions into the gas phase has focused attention on the linear Time-Of-Flight Mass Spectrometer (TOFMS). For a variety of reasons, including an unlimited mass range, high collection efficiencies and compatibility with pulsed ionization experiments, the TOFMS is the instrument of choice for performing these experiments. A major drawback of the TOFMS, particularly severe for LD experiments, is the relatively low resolution of the detected ion signals. Large distributions in the initial positions, velocities or times of ion formation may all lead to broadening of the detected ion signal and reduced signal resolution. Post Source Pulse Focusing (PSPF) is a method which has been proposed for improving the resolution of ion signals in a linear TOFMS [1, 2]. PSPF involves application of a compressing voltage pulse to a group of selected ions after they have been accelerated from the TOFMS source region using conventional electric fields. The only instrumental modification required is the addition of a separate voltage pulising region adjacent to the TOFMS acceleration regions.

A 2 m linear TOFMS has been constructed at this institute incorporating the necessary modifications for performing PSPF. A drawing of the source region design is shown in Figure 1. The source region consists of 2 adjustable static electric field acceleration regions and a 10 cm pulsed acceleration region. The pulsed acceleration field is defined by 10 plates spaced at 1 cm and a series of 10 1 kΩ resistors. The ion acceleration assembly is horizontally and vertically micrometer adjustable for maximizing the detected ion signals. In operation the ionizing laser fires and simultaneously triggers a digital delay generator and the 200 MHz data recording system. The ions created are accelerated through the source and secondary acceleration region. After an appropriate delay, determined by the highest mass ion to be focused, a voltage pulse is applied to the PSPF region. Under nominal conditions more than 80% of the mass spectra relative to the highest mass may be focused in the 10 cm region by the voltage pulse.

![Figure 1: Schematic representation of the source region design for a 2 m linear TOFMS incorporating a 10 cm Post Source Pulse Focusing region.](image-url)
Extensive theoretical modeling using the instrumental configuration described and a wide variety of ion formation conditions suggest that PSPF should allow significant improvement in the resolution of ion signals detected in the linear TOFMS, even when these ions are created with broad distributions in velocity or formation time. The ability of PSPF to compensate for the latter so called "start time errors" is a promising feature of this approach. In Figure 2 are displayed the typical unfocused and PSPF focused molecular ion signals for Zn Mesoporphyrin at m/z 656. The compound was introduced by 266 nm LD directly from the surface of the insertion probe. Clearly, PSPF produces a dramatic enhancement in ion signal resolution. In other initial experiments, similar results have been observed for gas phase and matrix assisted LD ion signals from smaller molecules. These results confirm the potential for PSPF to produce high resolution ion signals in a linear TOFMS.

**Figure 2:** The Zn Mesoporphyrin molecular ion signals at m/z 656 obtained by direct laser desorption at 266 nm. A) Static field operation without pulse focusing. The signal represents the sum of 20 laser shots. Resolving Power (50%) = 100. B) Molecular ion signal focused using Post Source Pulse Focusing. The signal represents the sum of 2 laser shots. Resolving Power (50%) = 1700.

**Literature**


**Acknowledgements**

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Using Time-of-Flight Mass Spectral Data to Deconvolute Unresolved Chromatographic Peaks

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Mass spectral detection in chromatography yields a three-dimensional data field (time, mass, intensity) which greatly enhances the ability to determine the presence of unresolved peaks, as illustrated in Fig. 1. Though the total-ion chromatogram (a) shows an unresolved doublet, analogous to two-dimensional (e.g., flame-ionization) detection, reconstructed ion chromatograms for masses unique to each compound (b) are resolved in time [1]. Thus it is most useful here to view chromatography/mass spectrometry data not as a series of mass spectra acquired over time, but rather as a collection of mass chromatograms acquired simultaneously.

Unfortunately, routine deconvolution of unresolved peaks and extraction of pure spectra for each component are often hindered by the inadequate spectral-acquisition rates available with scanning mass spectrometers. In addition, spectral "skewing" caused by the rapid changes in analyte partial pressure (i.e., the GC peak profile) during each scan interferes with normal spectral-deconvolution algorithms.

The problems of spectral-acquisition rates and spectral skewing are eliminated by the use of time-of-flight mass spectrometry (TOFMS) with time-array detection [2]. We have developed a set of deconvolution algorithms which analyze GC/TOFMS data to find the number of components, determine their retention times, and extract pure spectra for each component.

**Determination of Overlapping Peaks**

The intensities of those ions that correspond to any given compound eluting from the GC will rise and fall synchronously as the partial pressure of that compound changes in the ion source. We detect the presence of two or more components within a single chromatographic peak, then, by finding sets of ions that reach their maximum intensities at different retention times. We search each mass chromatogram, or reconstructed ion chromatogram (RIC), for local maxima and then tabulate, for each scan, the number of RIC's that reach their maximum intensities at that scan. This information is plotted in the form of a histogram to yield a "Peak-Position Plot" (PPP). The PPP indicates the number of compounds present and their corresponding retention times.

**Determination of Unique Masses**

For each compound located from the PPP, we must find a mass present in its spectrum but absent in the spectra of its nearest-eluting neighbors. Since a "unique mass" has its maximum intensity in the scan corresponding to the retention time, to identify unique masses we represent the intensities for a given mass \( m \) in the PPP scans prior to, at, and following the scan time of interest by \( I_{m,-1}, I_{m,0}, \) and \( I_{m,+1} \), respectively. The mass having the largest \( \Delta \) score, calculated as

\[
\Delta_m = \frac{2I_{m,0} - (I_{m,-1} + I_{m,+1})}{\max(I_{m,-1}, I_{m,0}, I_{m,+1})} \log I_{m,0},
\]

is chosen as unique for the corresponding compound. The log function discriminates against noise spikes.
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Extraction of Pure Spectra

We assume that the shape of a unique-mass RIC represents the true elution profile for the corresponding compound. If so, the degree to which this profile matches other RIC's is related to the intensity of those other masses in the desired spectrum. We calculate cross-correlations between the unique mass RIC and all other RIC's; these values are then re-mapped to yield factors ranging from 0 to 1 such that values for masses unique to adjacent components vanish, as illustrated in Fig. 2. The factors are multiplied by the corresponding observed intensities, acquired at the elution time, to yield the deconvoluted spectrum. In detail, we first normalize each RIC to unit vector length over the region of interest by using the formula

\[ N_{m,s} = \frac{R_{m,s}}{\left( \sum_{i=a}^{b} (R_{m,i})^2 \right)^{1/2}}, \]

where \( N_{m,s} \) is the normalized intensity for mass \( m \) at scan \( s \), \( R_{m,s} \) is the raw intensity, and \( a \) and \( b \) are the first and last scan numbers, respectively, for the desired scan range. Cross-correlations are then calculated between the normalized RIC for the unique mass of interest, \( p \), and all other masses, \( q \), as

\[ \text{Corr}(p,q) = \frac{\sum_{s=a}^{b} N_{p,s} N_{q,s}}{\sum_{s=a}^{b} N_{p,s}^2}. \]

We identify the cross-correlation between the RIC for the compound of interest and that for the unique mass of the nearest-eluting species as \( C_{low} \) and linearly re-map the correlation values as

\[ \text{Corr}'(p) = \frac{\text{Corr}(p) - C_{low}}{1 - C_{low}} \]

to produce the desired factors.

Performance

Representative results are shown in Fig. 3. The total-ion elution profile, PPP, and unique-mass RIC's for a pair of nearly-coeluting compounds, obtained from TOFMS data acquired at a rate of 30 spectra per second, are shown in Fig. 3a. The deconvoluted spectra, shown in Fig. 3b–c, are virtually identical to those of the pure species. Note that the two retention times differ by 0.067 s—that is, only two spectral acquisitions! We have obtained similar results in cases where one component was present in as much as a 50-fold excess.

![Figure 3. Total-ion chromatogram overlayed with PPP, and unique-mass chromatograms (a) for two overlapping compounds. Deconvoluted spectrum for benzene (b) and cyclohexane (c).](image)

1 For example, see J. E. Biller and K. Biemann, Anal. Lett., 1974, 7, 515-528.
High Speed Analysis of Complex Mixtures by Gas Chromatography / Time-Of Flight Mass Spectrometry

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In normal high resolution gas chromatography/mass spectrometry (GC/MS), the components of a mixture are separated chromatographically and then detected using a mass spectrometer. The key to this technique is the chromatographic separation—while the mass spectrometer serves to detect and identify the components. In cases where components elute nearly simultaneously from the chromatographic column, pure mass spectra (and thus identification) of the coeluting species may be obtained using deconvolution techniques. The resolving power of deconvolution depends on a mass spectral acquisition rate adequate for an accurate reconstruction of the elution profile. A typical scanning mass spectrometer only acquires a few spectra across the elution band of a component from a capillary column. Under these conditions, the shape of the elution profile cannot be determined, and the resulting mass spectra are skewed due to changes in the pressure of the analyte in the ion source during a single scan. In some cases, the scan rate can be increased, but at the cost of diminished sensitivity. With low capacity narrow-bore columns, the attainable mass spectral acquisition rate is limited by the need to maintain a detectable amount of analyte for each scan.

The combination of time-of-flight mass spectrometry (TOFMS) and time-array detection (TAD) allows spectral acquisition rates and sensitivities compatible with the chromatographic timescale, even when capillary columns are used [1]. In our instrument, which was designed and initially constructed by H. Wollnik et al. [2] and modified for GC/MS at Michigan State University, ions are made continuously and a portion of them are stored in the ion source. Every few hundred microseconds, these ions are pulsed out of the source, accelerated, drifted through a 1 m flight tube to a grid-free ion mirror and are reflected back to a detector. Each of the transient waveforms produced from the detector contains unskewed spectra data over the full mass range with unit resolution. A selected number of successive transients are summed to form each recorded mass spectrum. Up to 50 mass spectra are produced each second, allowing accurate determination of retention times and elution profiles. When two or more compounds coelute, mass spectral deconvolution can determine the presence of coeluting species and then provide an accurate retention time and pure mass spectrum for each compound [3].

The ability to perform effective deconvolution of the GC/MS data reduces the need to chromatographically resolve the components of a complex mixture. Thus, the analysis time can be reduced without sacrificing any information. We have reduced the chromatographic analysis time by shortening the chromatographic column and increasing the linear velocity of the GC carrier gas to provide the optimal combination of theoretical plates and analysis speed. Narrow-bore columns provide better resolution when operated under these conditions.

When this approach is applied to a 60-component mixture of volatile organic contaminants (VOC), the run time is reduced from 30 minutes to 80 seconds. Figure 1 shows the total ion chromatogram (TIC) for an 80 second analysis. Although a portion of the chromatographic resolution has been sacrificed, many of the components of this mixture still elute separately. Figure 2 shows an expansion of a region of the TIC with a Peak Position Plot (PPP) [2] from the deconvolution overlayed. The times when the elution profiles of a group of ions reach their maximum intensity (retention time) is displayed in this plot. A total of 22 compounds were located and identified in this 14 second region. Visual inspection reveals the presence of some compounds as irregularities in the shape of the elution profile, but species whose retention times differ by less than 0.1 s may not be noticeable. The PPP allows precise location of these compounds, even in an unknown sample. Pure mass spectra have been extracted for each of these compounds and they compare favorably with library spectra.

This approach reduces the GC/MS analysis time of complex mixtures with known or unknown composition by a factor of 10 or more without sacrificing any analytical information. The combination of TOFMS/TAD and mass spectral deconvolution can follow faster chromatographic technologies as they emerge, shortening analysis times even further.

Figure 1

![Figure 1](image1.png)

Figure 2

![Figure 2](image2.png)
A Time-of-Flight Mass Spectrometer for High-Speed Chromatography

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The decreasing analysis times of modern separation methods have placed increasingly stringent demands on the temporal responses of injection, detection, and data acquisition systems. In particular, the mass spectral acquisition rates available with conventional gas chromatography/mass spectrometry (GC/MS) systems are not adequate for the sub-second peak widths achieved with high-resolution GC columns. To overcome this difficulty, we have developed a time-of-flight mass spectrometry (TOFMS) system for use with high-speed GC.

In order to serve as a useful detection system, the TOFMS instrument must have the following characteristics: a mass range 1-1000 u; unit mass resolving power throughout the entire mass range in each transient; acquisition of at least 30 spectra per GC peak; efficient ion storage between extractions; and source conductance adequate to maintain the integrity of narrow elution profiles. To achieve these goals, our system incorporates a novel source providing efficient ion generation, storage, and extraction as well as an open geometry for high conductance; an inhomogeneous-field ion mirror for mass-independent lateral and longitudinal ion focusing; an integrating transient recorder (ITR) for time-array detection of complete TOFMS transients. The flight tube, source, and mirror were originally designed and built by Wollnik and coworkers, and the ITR was designed at Michigan State University. A modified source, built at Michigan State, was designed to allow operation under GC conditions.

A dual multichannel plate (MCP) detector, designed and constructed by Wollnik and coworkers, uses two Galileo MCP plates mounted in a single, low-profile unit. Controllable voltages are applied to each surface of the plates. Current-to-voltage conversion is accomplished by a Comlinear E-220 amplifier. Time-array detection was performed with an ITR having 8-bit analog-to-digital converter (ADC) resolution and 5-ns time resolution. Transients are typically generated at 5 kHz and are digitized by the ADC. A user-specified number, n, of successive transients are summed to yield a mass spectrum with enhanced signal-to-noise ratio (S/N) and dynamic range. The spectral generation rate is equal to 5000/n.

A single transient obtained from analysis of residual gas is shown in Figure 1a; the sums of 2, 20, and 200 transients are shown in Figures 1b-d. The ionization, ion storage, and transmission efficiencies in the instrument are so high that as few as 20 transients must be summed to provide an adequate S/N. This leads to a potential spectral-acquisition rate of 250 spectra per second—more than adequate for capillary-column GC analyses. GC/MS data from mixture of hexanes is shown in Figure 2a. These data were acquired at the rate 100 spectra per second; yet the representative spectrum, shown in Figure 2b, is clearly recognizable.

Figure 1. Single and summed transients.

Figure 2. TIC (a) and representative spectrum (b) of hexane mixture obtained at 100 spectra per second.
Characterization of Storage Electron Ionization Sources for Time-of-Flight Mass Spectrometry

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In GC/MS analysis, the analyte is continuously delivered to the source of the mass spectrometer. Thus, in a time-of-flight (TOF) mass spectrometer using pulsed extraction, the highest sensitivity is achieved with continuous ionization and storage of the ions between pulsed extractions. The novel source design of Wollnik, et al. 1 provides both high sensitivity and mass resolution across the entire mass range. Since the storage efficiency of such a source is directly related to sensitivity, it is important to understand and optimize the storage process.

To characterize the storage performance of the source, a model of ion behavior has been developed. We assume that the number of ions in the storage region, n, follows the rate equation \( \frac{dn}{dt} = k_F n - k_L n \), where t is time, and \( k_F \) and \( k_L \) are the formation and loss rate constants, respectively. Integrating Eq. 1 leads to the expression \( n(t) = \frac{k_F}{k_L} (1 - e^{-k_L t}) \). Thus, we expect the ion population to rise exponentially to a limit given by \( k_F / k_L \), and with a time constant given by \( 1 / k_L \) (see Figure 1). The longer the time constant is for the exponential approach to steady-state the greater the ion storage efficiency. The greater the value of \( k_F / k_L \), the greater the sensitivity.

Observations have indicated that alteration of the source geometry can significantly affect the storage capability. In order to identify the parameters critical for ion storage, we have systematically studied six different source geometries. For each source geometry, the ion storage effectiveness was evaluated in terms of the ion storage time constant. The time constant was obtained by fitting the theoretically derived equation for ion population (2) to the peak intensities (m/z = 44) at many source extraction periods. The maximum mass resolution and the formation rate constant were also measured for each configuration.

Tabulated below are the different geometries tested and their results. (See Figure 2 for a generic source design.)
Extracted ion volumes that are essentially field free (geometries having cages: A-D) are more efficient at ion storage and achieve better resolution. The production of a dense electron cloud appears to be critical to ion storage, (geometries A and D which have opposing linear filaments and geometry B which has a large circular filament). In addition filament alignment, often difficult to control, had a significant impact on the source storage efficiency.

Experiments have indicated that the storage of helium ions under GC conditions greatly decreases the number of analyte ions which can be stored in the source. Modeling shows that the application of a sine wave of the correct amplitude and frequency to one of the source grids causes low mass ions to be accelerated from the source storage region. High mass ions, on the other hand are not removed.

Comparison of the residual gas spectra obtained with and without the application of a 2MHz, 30Vpp "tickle" voltage, (see Figure 3a-b) reveals that up to 10 times more high mass ions are stored when the helium ions are continuously removed.

Figure 3a. Spectrum obtained without the tickle applied.

Figure 3b. Spectrum with the tickle voltage applied.

Comparison of storage time constants (1/kL) obtained with and without the application of the tickle voltage, (0.77 s and 0.27 s respectively) indicates that the analyte ion loss rate is substantially decreased when the helium ions are being selectively removed. (see Figure 4)

Figure 4. Effect of applying a tickle signal on the storage curve for analyte ions.

The capability of comparing a nitrogen chromatogram generated from a gas chromatograph (GC, Varian model 3400) linked to a thermal energy analyzer (TEA, Thermedics Inc. Model 610) with a total ion chromatogram (from a Finnigan-MAT Ion Trap Mass Spectrometer, ITMS) has provided a new means to screen and identifying trace levels of nitrogen-containing compounds in complex mixtures. Prior to the work described here, it has not been possible to simultaneously acquire TEA and MS data. What was needed was a viable GC-TEA/ITMS interface to combine the capabilities of both instruments.

INTERFACE: The TEA pyrolyzer is installed vertically on the GC oven box. A hole was drilled through the GC oven to insert the pyrolyzer transfer line. At the exit of the GC capillary, a glass "Y" connector with a split ratio of 1:1 was attached. Two six inch guard columns were attached to the fork side of the connector. The ends were then connected to two separate, low dead volume valves (MCV-1/100 control valve, Scientific Glass Engineering PTY. LTD). The control valves were placed in a specially machined heating block. A heating cartridge in the center of the heating block provided stable heating at variable temperatures (utilizing the alternate heater controller from the GC). The exit of one valve was connected to the ITMS interface (via capillary guard column), the other to the TEA interface. A complete schematic of the linked GC-TEA/ITMS system is shown on Figure 1. The GC carrier gas linear velocity did not change with respect to the ITMS when operating in GC-TEA/ITMS mode. Data acquisition for the TEA utilized a MLS A/D data acquisition card (Industrial Corporate Source, San Diego, CA) and a stand-alone PC. A variety of unique data manipulation software programs were designed to offer the user a full range of processing options.

RESULTS: The capability of the GC-TEA/ITMS to screen for and identify nitrogen-containing compounds is illustrated Figure 2. The peak retention times of only nitrogen-containing compounds from the TEA chromatogram (Figure 2A) have a corresponding peak in the TIC (Figure 2B). Explosives in a complex matrices can be seen easily with GC-TEA/ITMS (Figure 3). Figure 3A demonstrates how a sample of diesel fuel was screened for explosives using the TEA chromatogram. The TEA response for TNT can be confirmed by locating the corresponding peak in the TIC (by retention time, Figure 3B) and extracting the mass spectrum of TNT (Figure 4B inset). Once the nitrogen compounds are identified, and if there are several samples of this type, a MS/MS scan function can be generated for the target compounds. For example, single reaction monitoring (SRM) of TNT (Figure 3C inset), [fragmentation of m/z 210 (precursor ion) to m/z 164 (product ion)], removes all superfluous peaks contributed by diesel fuel from the TIC (Figure 3C). This clearly shows the analytical power of ITMS for linked GC-TEA/MS/MS.

Another application of this new system is its use for drug detection. Figure 4 illustrates how phencyclidine (PCP) can be detected (Figure 4A) and identified (Figure 4B inset) in marijuana extract. In addition to PCP we also identified 1,3-benzothiazole, a rubber product residue. A strong 1,3-benzothiazole TEA response (Figure 4A) facilitated the location of 1,3-benzothiazole on the TIC for mass spectral identification.

We have not attempted to perform quantitative studies or optimize detection limits for specific compounds. We are addressing these issues currently. Our results indicate that linking TEA with mass spectrometry will provide a new analytical tool to screen and identify trace levels of nitrogen compounds.

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Figure 1

Figure 2

Figure 3

Figure 4

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Supercritical Fluid Chromatography-Mass Spectrometry

Using a Quadrupole Mass Filter/Ion Trap Mass Spectrometer

with External Ion Source

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Supercritical fluid chromatography (SFC), with its complementary nature to gas chromatography (GC) and high performance liquid chromatography (HPLC), has become an increasingly widespread method of analysis over the past few years. For example, SFC has been applied to mixtures which are too low in volatility or thermal stability for GC, or which lack a good functional group for sensitive HPLC detection. Compilations of SFC applications have been published.

Historically, most detection for SFC has been performed with a flame ionization detector. As the routine use of SFC has grown, so has its combination with detectors which provide more informative knowledge of the analyte. Such is the case, for example, in supercritical fluid chromatography-mass spectrometry (SFC-MS). As the field has developed, many researchers in SFC-MS have moved to mass spectrometers that are able to capitalize on some of the inherent advantages of SFC. In particular, a move to higher mass range instruments, either quadrupoles, double-focusing sector, or Fourier-transform and more recently the Paul ion trap has been apparent. However, the quadrupole ion trap has the potential to deliver the advantages of high performance instruments without the disadvantages of being generally large, complex, and expensive.

Recently, we described a tandem quadrupole mass filter/ion trap mass spectrometer. Ions produced in a conventional ion source are transmitted from the quadrupole, with or without mass selection, into the quadrupole ion trap where they are further characterized. The combination of capillary SFC with an instrument allowing external ionization (see Figure 1) provides a number of distinct advantages. The instrument provides good sensitivity, the capacity for performing MS/MS experiments, traditional electron and chemical ionization, and it tolerates relatively high flow rates of mobile phase from the chromatograph.

The instrument was characterized and optimal experimental conditions determined with perfluorotributylamine, anthracene, and methyl docosanate. A sample of 80 femtomoles of anthracene provided a S/N of 20 (see Figure 2 and 3). Once the operating parameters were optimized, two less volatile mixtures were characterized. The first was a standard, 770-average-molecular-weight poly(dimethylsiloxane). The second was a derivatized surfactant for which the full structure was not known (see Figure 4), but was determined with the SFC-MS data.

References

Figure 1. Schematic diagram of the SFC-Q/Trap mass spectrometer

Figure 2. Mass chromatogram and mass spectrum for the protonated molecule of anthracene for 780 fmoles obtained under methane CI conditions.

Figure 3. Mass chromatogram for 78 fmoles of the protonated molecule of anthracene.

Figure 4. Reconstructed-total-ion-chromatogram of a derivatized alkyl-ethoxy-sulfate after derivatization with diazomethane obtained under ammonia-CI conditions.
INTRODUCTION
Collisional activation (CA) has become a very useful technique in tandem mass spectrometry for
inducing the characteristic dissociations of selected ions for fundamental and structural studies. The
principal advantages of high-energy CA lie with its generality and efficiency. Its versatility is manifest
in the ability to generate useful MS/MS data for almost all ionic species up to 2500da, including
biomolecules, without the need of constant instrumental optimization.

INSTRUMENT
The VG ZAB-T is a four-sector mass spectrometer of BEBE layout in which MS-II is of special
design featuring a wide-acceptance inhomogenous-field ESA coupled with either a conventional or a
six-inch microchannel plate (MCP) detector. The MCP detector, while not employed in the
experiments reported here, affords much greater efficiency in the generation of MS/MS spectra from
limited amounts of materials than does a conventional detector.

As a four-sector instrument, the ZAB-T makes possible the selection of precursor ionic species and
subsequent analysis of fragment species with greater than unit resolution in the same experiment, a
capability not possible on our three-sector instrument of EBE design. Such selectivity allows the study
of competing fragmentation pathways whose products would not be resolved otherwise. At the same
time, essential reproducibility of spectra between the two machines is maintained.

The wide energy acceptance of the ESA of MS-II coupled with a flexible collision cell between MS-I
and MS-II facilitates the generation of MS/MS spectra with varying collision energy without the
sacrifice of resolving power.

By using the opportunities afforded by the ZAB-T, we have begun reinvestigating prototypical systems
that exhibit charge-remote fragmentation. These systems were chosen because of the potential in
determining the nature of long hydrocarbon chains on molecules and because that capacity may be
enhanced by elevated high-energy CA.

EXPERIMENTAL
All experiments were carried out on the ZAB-T. Precursor ions were desorbed by Cs+ LSIMS,
accelerated to 10keV, and selected by MS-I with resolution >1000. CA was performed in the
collision cell between MS-I and MS-II with He as the target gas at 50% beam suppression. Collision
energy was 10keV except for the collision energy study in which the cell was floated to the
appropriate potential. Fragment ions were analyzed in MS-II with greater than unit resolution and
detected at the conventional detector.

RESULTS AND DISCUSSION
Collision Energy Study
CA at a variety of energies (lab frame) from 10keV down to 500eV were performed on the
n-dodecyltrimethylammonium ion. The intensities of the eight charge-remote losses (m/z 212 to m/z
116) and the major charge-related fragmentations (m/z 100 to m/z 41) were separately summed and
the sums were ratioed. The ratio of charge-remote/charge-proximate as a function of collision energy
is shown by graph in figure 1.

Since charge-remote fragmentation carries more structural information than charge-proximate
cleavages, the ratio of charge-remote/charge-proximate is a measure of information content for the
MS/MS spectra. Based on the energy available in the center-or-mass frame of reference, it is likely
that the high collision energy response which is increasing would prove to be more general than the
lower energy maximum and thus motivate the use of elevated collision energies for this class of CAD.
At 500eV collision energy, the charge-remote fragmentation for this species disappears except for the
equivalent loss of methane.

Mechanism
The MS/MS spectra showing charge-remote fragmentation of 7,7,8,8-d4 palmitic acid is shown in
figure 2. The pattern of fragmentation in this more detailed spectrum is still consistent with the
proposed 1,4-H2 loss resulting in C-C bond cleavage.1 The cleavages labelled a-e show the expected
progression in m/z. The intensities of b and c are depressed because of a proposed 1,4-HD
elimination in these cases. The significant minor peaks at m/z of 142, 144, 159, and 173 may be due
to mechanistic complications or some inaccurate labelling in the precursor.

Structural Determination
MS/MS spectra featuring charge-remote fragmentation were obtained for a series of long-chain fatty
acids (stearic, oleic, linoleic, linolenic, arachidonic, etc) and for a set of EETs (monoepoxy
eicosatetraenoic acid isomers derived from arachidonic acid). The spectrum for the 14,15-EET is
shown in figure 3.
Improved resolution revealed details previously obscured in MIKES spectra in the MS/MS spectra of these long-chain fatty acids: low-mass radical-ion series of formula \( \text{CH}_2(\text{CH}_2)_n\text{CO}_2\text{Li}_2^+ \) \((n=0, 1, \text{etc})\) which parallels the stability trends known for distonic ions (rapidly diminishing abundances for \( n>2 \)),\(^2\) and the splitting of peaks due to allylic and certain allylic-vinyl cleavages into doublets with the peaks corresponding to the closed shell ionic species dominant. The spectra are otherwise consistent with MIKES spectra and show the gaps in the peak patterns corresponding to the positions of double bonds\(^3\) and the prominent peaks corresponding to cleavage sites that are allylic to the proximate double bond and vinyl to the distal double bond of a homoconjugated pair.\(^4\)

For the EET's, the features corresponding to charge-remote fragmentation are present in the MS/MS spectra of these compounds and are useful in structural assignments even though they are not the most prominent features. Most prominent are the four major sequential peaks that correspond to cleavages about the epoxy group. The epoxy related cleavages are much enhanced over those for a simple long-chain epoxy acid. Also prominent are peaks corresponding to water loss and the low-mass radical-ion series.

**CONCLUSIONS**

The ZAB-T shows great promise as a tool for structural and fundamental studies. The range of molecular weights it can handle should make it useful for studying molecules of biological interest.

**REFERENCES**

The use of analytical instruments on manned spacecraft has been limited by the lack of sufficiently compact, space-qualified devices with the required specificity and sensitivity. Only recently, longer manned missions have been achieved on space shuttle operations and consequently, methods for analytical measurements of the spacecraft environment are of importance. For example, a rapid and routine method for monitoring the spacecraft environment for contaminants, is essential. To address this need as well as support research on board the spacecraft, we have designed a compact triple-sector MS/MS instrument, with an EBE configuration.

Several constraints are placed on the analytical mass spectrometer for operation in space applications. The three primary constraints are: power, size, weight and reliability. The EBE design constraints (which become design goals) are: (1) power consumption of less than 100 Watts, (2) less than 100 pounds, and (3) less than 2 cubic feet in size. Unfortunately, these constraints limit the mass range, resolution, and utility of various types of spectrometers. For example, a scanning magnet or a quadrupole were ruled out due to their excessive power requirements.

We have redesigned the already space-proven mass spectrometer used on the NASA Mars lander mission. This mass spectrometer is an accelerating voltage scanned mass spectrometer with a Nier-Johnson configuration. Modification of the source has been accomplished for improved transmission and simplified construction. This ion source is a hybrid of the original source and a source design of Matsuda, et al.\(^1\) employing an acceleration/deceleration region. The original and the modified sources were compared via modelling with SIMION under optimum focusing and extraction conditions for a given set of initial parameters. Comparing the off axis velocity and spatial divergence values, it is estimated that the new source is an order of magnitude more efficient.

Analysis of the instrument configuration revealed that the image and object distances as well as the shunts for the electric sector were accurate\(^3\). The image exit slit half-angle was calculated to be 3.7\(^\circ\).

The design of MS2 involves a lens system that will refocus the diverging beam exiting MS1 on to a microchannel plate (MCP) and allow control of the collision energy. This surface dissociation technique has been used successfully by Aberth\(^7\). The MCP has a length to pore ratio of 40 and a tilt of 1\(^\circ\) yielding an effective ion interaction with approximately 1100 pores.
A lens system system for collecting the resulting daughter ions is currently being designed however, the required vacuum system, power supply, and control circuitry have been designed. A diagram of the complete spectrometer in a custom vacuum housing is shown in Figure 1. MS1 has a fixed magnet and MS2 has a scanned electric sector (floated relative to MS1) for daughter ion analysis.

The power consumption is less than 250 Watts. The weight of the spectrometer is less than 100 pounds and it is approximately 2 cubic feet in size. The resolving power is 375 and currently employs fixed slits.

This project is supported by a NASA small business innovative research grant #SBIR 87-1-II

PERFORMANCE OF AN ATMOSPHERIC SAMPLING GLOW DISCHARGE IONIZATION SOURCE WITH A TRIPLE QUADRUPOLE MASS SPECTROMETER

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Introduction

Atmospheric sampling glow discharge ionization (ASGDI) has been shown to be a highly sensitive ion source for the detection of organic compounds in air (1,2). We first interfaced this ion source with a home-built quadrupole/time-of-flight (QT) instrument (3). A second MS/MS instrument, a Finnigan ion trap mass spectrometer (ITMS), was then modified extensively to adapt the ASGDI source (4). We have recently interfaced an ASGDI source with a Finnigan TSQ 700 tandem quadrupole instrument. Part of the rationale behind this work was to adapt ASGDI in a very simple and inexpensive manner to a widely available state-of-the-art beam-type tandem mass spectrometer. In particular, we are interested in evaluating the ASGDI/TSQ 700 combination as an explosives vapor detector.

TSQ 700 Modification

An ASGDI ion source geometry was designed that would adapt directly to the TSQ 700 ionization region vacuum manifold. The modifications to the TSQ 700 include:

- removal of the normal EI/CI ion source and solids probe flange and replacement with the ASGDI source
- addition of a 1 cm tube extension to the first lens element for ion extraction
- addition of a 10 L/s roughing pump to evacuate the ASGDI source to 0.8 torr
- addition of a DC power supply to provide the 400 V, 10 mA for the discharge

Results and Discussion

An important application of ASGDI is the ionization of trace quantities of high explosives in air. We have therefore focussed our attention first on the negative ion performance of the ASGDI/TSQ 700 system. The negative ion mass spectra obtained with the ASGDI/TSQ 700 instrument are virtually identical to those obtained with the other instruments. The MS/MS spectra show qualitative differences, however. The major difference between the TSQ 700 and the QT is much poorer product ion resolution in the latter. Mass resolution is comparable for the TSQ 700 and the ITMS but collisional activation conditions are significantly different for the trapping instrument vs. the beam-type instruments. The major difference in the product ion spectrum is the relative intensity of the NO$_2^+$ product ion. This product ion can be the major ion in the beam-type instruments but is essentially absent in the ITMS spectrum.

The appearance of a large NO$_2^+$ in the MS/MS spectra acquired with the beam-type instruments can be useful in rapid screening for nitroaromatics via the "targeted product ion" mode of operation (5). In this mode, the first quadrupole is operated as a high pass filter (e.g., passing only ions of m/z > 150) and scanning the second analyzer to detect any product ions characteristic of a compound class. The product ions NO$_2^+$ and NO$_3^+$, for example, are characteristic of nitroaromatics and nitrate esters, respectively. It is therefore desirable to maximize NO$_2^+$ from the MS/MS of M of TNT for application of the targeted product mode of operation. The use of argon as a collision gas at pressures sufficient to attenuate the parent ion signal by 50 % and use of a laboratory collision energy of about 40 eV constitutes the best compromise between transmission and NO$_2^+$ production.

The performances of the three ASGDI/tandem mass spectrometers were compared under different MS/MS operating modes. In all cases an equal and constant flux of TNT was admitted into the instrument (maximum concentration estimated to be 1 ppb) and data were collected for two seconds. This normalized the analyte quantity sampled by each instrument. A quantitative measure of "signal/noise" for each
experiment was taken as the analyte signal (minus background signal) divided by three times the standard deviation of the background signal. For the beam-type instruments, the NO$_2$ product ion was used as the analyte signal whereas the product ion at m/z 210 was used for the ITMS data. Table I summarizes the "signal/noise" results acquired under various MS/MS operating modes. The various operating modes establish compromises between signal/noise and specificity via the normal trade-offs between resolution and transmission, duty cycle and scan length, etc. The most direct comparison to evaluate signal/noise at equal specificity comes from the data acquired with MS I resolution > 400 and MS II resolution maximized. For the TSQ 700 and the ITMS, MS II resolving powers are comparable. However, the resolution of MS II of the QT is at least an order of magnitude lower. The TSQ 700 provides slightly better signal/noise than does the QT and with much superior specificity. The ITMS, however, provides roughly an order of magnitude greater signal/noise with specificity at least as good as that of the TSQ 700. At the cost of specificity, however, the beam-type instruments can come closer to the performance of the ITMS. This is apparent in the numbers obtained using various forms of parent ion resolution degradation, including the targeted product ion mode. The TSQ 700 can approach the performance of the ITMS in the targeted product ion mode with a narrow scan (to improve duty cycle) over the m/z 46 product ion. An analogous procedure is not available for the QT.

Conclusions

The TSQ 700 can be fitted with an ASGDI source simply and with minimal expense (hardware costs < $10k). The ASGDI/TSQ 700 provides performance superior to that of the QT instrument both in terms of specificity and signal/noise (which should translate to lower limits of detection). The ASGDI/ITMS provides the best performance of the three instruments in terms of specificity and signal/noise. However, at degraded specificity the ASGDI/TSQ 700 can provide performance in MS/MS signal/noise comparable to that of the ASGDI/ITMS.

Research sponsored by the Federal Aviation Administration


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<th>OPERATIONAL MODES</th>
<th>TSQ700</th>
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<tr>
<td>m/Δm&gt;400</td>
<td>m/Δm&gt;400, Full Scan</td>
<td>30</td>
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<tr>
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<td>m/Δm=50, Full Scan</td>
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<td>20</td>
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<tr>
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<td>m/Δm&gt;400, Full Scan</td>
<td>75</td>
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<tr>
<td>RF only, m/z&gt;180</td>
<td>m/Δm&gt;400, Narrow Scan</td>
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Table I
The field of ion-molecule chemistry is well established at relatively low pressures (<10 Torr) with thousands of rate coefficients having been measured. Conversely, of the few data that exist at pressures up to and exceeding atmospheric pressure, only a small fraction are quantitative. Studies of ion-molecule reactions under the collision dominated conditions at atmospheric pressure can provide a wealth of fundamental information with valuable application to environmental chemistry and trace analysis.

We are currently developing an ion mobility spectrometry (IMS) system which will allow measurement of ion-molecule reaction rate coefficients at atmospheric pressure and over a range of temperatures from ambient to 200°C.

IMS is a method of separating ionic species according to their drift velocity (or more correctly their mobility) through a neutral gas under the influence of an electric field and has been used to date essentially as an analytical tool. The present IMS is illustrated in Figure 1 and consists of a drift tube approximately 37 cm long and 9 cm diameter. Ions are produced by a 15 mCi $^{60}$Ni source and drift through a counter flowing drift gas, under the influence of an electric field towards the Faraday plate detector. Ions are admitted to the drift region in short bursts by pulsing the Bradbury-Nielson grid, then they are collected at the Faraday plate, amplified and recorded by a signal averager.

![Figure 1. The Ion Mobility Spectrometer](image)

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<td><strong>DRIFT GAS</strong></td>
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To study ion-molecule reactions, the drift gas is seeded with known concentrations of a reactant gas and the resultant modification of the ion arrival time spectrum can be modelled to obtain the rate data.

We report here two preliminary negative ion reaction studies under the IMS conditions given in Table 1:

1. Azulene in the source gas (to produce Az$^-$) and CF$_3$Br in the drift gas; shown in Figures 2A, 2B and 2C. The product ion has a higher mobility than the reactant ion. Figure 2D shows the spectrum obtained with CF$_3$Br in the source gas and pure $N_2$ as the drift gas. A pulsed e-beam high pressure mass spectroscopy (PHPMS) study of the reaction between Az$^-$ and CF$_3$Br revealed the product ion to be Br$^-$ and a rate coefficient $8.2 \times 10^{-10}$ cm$^3$ s$^{-1}$ is reported at 125°C in 3 Torr of Methane [1].

2. CFCl$_3$ in the source gas (to produce Cl$^-$) and CH$_3$Br in the drift gas; shown in Figures 3A, 3B, 3C and 3D. The product ion has a lower mobility than the reactant ion in this case. The reaction between Cl$^-$ and CH$_3$Br has been well studied and the product ion is known to be Br$^-$. From the PHPMS results of Caldwell et al. [2] a rate coefficient of $8.8 \times 10^{-12}$ cm$^3$ s$^{-1}$ can be determined at 125°C in 5 Torr of Methane.

The observed ion arrival time profiles are in agreement with those predicted by the analytical model of Woo and Whealton [3]. Thus with the ensuing addition of a mass spectrometer, reactant gas handling plant and source handling plant we will be able to quantitatively study many chemical systems of interest at atmospheric pressure.

REFERENCES
Figure 2. Ion mobility spectra with Azulene in the source gas and (A) 0 ppb, (B) 5 ppb and (C) 50 ppb CF$_2$Br$_2$ in the drift gas. (D) is the spectrum obtained with CF$_2$Br$_2$ in the source gas and pure N$_2$ drift gas.

Figure 3. Ion mobility spectra with CFCl$_3$ in the source gas and (A) 0 ppb, (B) 100 ppb, (C) 150 ppb and (D) 300 ppb CH$_3$Br in the drift gas.
GAS-PHASE ION CHEMISTRY USING A TROCHOIDAL ELECTRON MONOCHROMATOR

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A trochoidal electron monochromator/mass spectrometer has been constructed to perform environmental analyses for which it is ideally suited for such applications. The kinetic energy of the electron beam can be easily varied from 0.025 to 30 eV under computer control. No reagent gas is used to moderate the electron energy; consequently, the mass spectra are expected to be free from artifacts which arise from ion/molecule interactions and that vary with the nature of the analyte, and ultimately lead to spectral irreproducibilities (Laramee, 1986). The instrument can be applied to the solution of environmental problems which cannot be reached by other simple mass spectrometric techniques such as isomer differentiation of tetrachlorodibenzodioxins, polychlorodibenzofurans, and polynuclear aromatics. Although positive electron affinities can be measured by several techniques, an electron monochromator/mass spectrometer system is the only instrument for measurement of negative electron affinities. It can be used to investigate a wide range of chloro/fluro aliphatics. These compounds are responsible for the observed loss of O³ in the Antarctic vortex (Anderson et al., 1991).

The trochoidal electron monochromator was first brought to practice by Stamatovic and Schultz, 1970, and has since been used by several investigators (refs. in Christophorou, 1984). All electron optic components are made of 99.999% pure molybdenum. Non-magnetic stainless steel is used for the other vacuum system components. Electron scattering resonances for molecules which possess negative electron affinities are detectable since all scattered electrons have their axial velocity vector reoriented and do not have sufficient axial momentum to overcome a retarding barrier. Molecules with positive electron affinities which can form stable anions with respect to autodetachment are mass analyzed and detected (Figure 1).

The operating principle of the electron monochromator/MS is electrons emitted by a rhenium filament are confined by a magnetic field and analyzed by crossed electric and magnetic fields. This arrangement produces intense electron beams (~3 x 10⁷ A) at very low energies (0.025 eV). Electrons entering the crossed field off-axis move trochoidally towards the center of the end-cap electrode with a drift velocity of E x B/B². Excellent peak shape, determined by third and fourth moment analysis is obtained which compares well with the instrument function. Isotope measurement accuracy is excellent at ±1.2% for the 99% confidence level, and the system can be operated over a wide range of sample pressures without loss of energy resolution for an energy spread of ±0.28 eV. The highest energy resolution obtained thus far is ±0.07 eV.

We have begun to test the hypothesis that positional isomers can be distinguished on the basis of differences in their electronic manifolds which can be accessed by discrete energy electrons. The isomers 1,2,3,4-tetrachlorodioxin and 1,3,6,8-tetrachlorodioxin show differences in the electron energy (e_max) needed to produce the maximum amount of M'' (Figure 2). These attachment energies follow the same ordering as their calculated lowest unoccupied orbital energies, ca. 0.96 and 1.59 eV, respectively. Tetrachloronaphthalenes (Halowax) are another class of environmentally relevant compounds which are used in electrical insulating materials, cutting oils, textiles and paper. Although their mass spectra are identical, the isomer 1,4,6,7 tetrachloronaphthalene reveals an e_max of 0.34 eV, while 1,4,6,8 tetrachloronaphthalene has e_max equal to 0.50 eV. The technique of monoenergetic electron capture ionization indeed appears to be sensitive to isomer position as these two isomers differ by a single chlorine from the β-position to the α-position. These resonances in ion production with respect to electron energy are unique to each compound and furthermore, it is unlikely that the same resonances will be found for the same mass ions. For this reason, monoenergetic electron capture ionization should be useful for the analysis of environmental compounds (Watson, 1990).

Multiple resonances to form a stable M'' radical anion at m/z 123 from nitrobenzene were observed at 0.06, 3.3, and 6.9 eV. The two higher energy peaks could be formed from independent electronic states or different ion structures of the same mass. The mass spectrum of heptachlor obtained by broadband (0-3 eV) electron capture from the electron monochromator/MS system was compared to the spectrum obtained from the Finnigan 4023 mass spectrometer. A factor of 10 increase in relative abundance for the higher mass ions was observed using the electron monochromator (Figure 3).
ACKNOWLEDGEMENTS

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REFERENCES

THE 39TH ASMS CONFERENCE ON MASS SPECTROMETRY AND ALLIED TOPICS

ANALYSIS OF POLYCYCLIC AROMATIC COMPOUNDS BY SUPERCRITICAL FLUID CHROMATOGRAPHY-MASS SPECTROMETRY USING ATMOSPHERIC PRESSURE CHEMICAL IONIZATION

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The analysis of polycyclic aromatic compounds (PAC) is of considerable importance due to concerns over the mutagenicity and carcinogenicity of many of these compounds. Because of the complexity of most PAC contaminated samples, methods of analysis that offer good chromatographic resolving power and selective and sensitive detection are required.

Supercritical fluid chromatography (SFC) is a complementary technique to both gas and liquid chromatography, which provides efficient separations of less volatile, higher molecular weight compounds with relatively short analysis times [1]. The difficulties in coupling SFC with mass spectrometry (MS) have been largely due to the pressure requirements of conventional mass spectrometers.

Atmospheric pressure ionization (API) MS is rapidly becoming a universal detection system for the separation sciences [2]. A rugged API-MS interface for the routine coupling of microbore packed-column SFC with a SCIEX API III triple quadrupole mass spectrometer has been developed [3]. The improved interface was designed to accommodate commercially available fused silica restrictors.

The atmospheric pressure chemical ionization (APCI) of PAC has been found to involve both charge and proton transfer reactions. The ratio of M⁺ to [M+H]⁺ ions will depend on the ionization energies and proton affinities of the analyte. Figure 1 illustrates that as the size of polycyclic aromatic hydrocarbons (PAH) increases, proton transfer becomes the predominant form of ionization. This reflects the increase in proton affinity with increasing PAH molecular weight. Nitrogen containing PAC (e.g. carbazole) produced predominantly protonated molecules while oxygen and sulphur heterocycles produced molecular ions.

The addition of chemical ionization modifiers to the make-up gas entering the source can be used to alter the reagent ion composition in the APCI plasma and therefore dictate the type of spectrum obtained. Benzene was used to promote charge transfer since trace compounds that have lower ionization energies than benzene, which is true for all higher molecular weight PAH, will readily transfer an electron to benzene radical cations. Proton transfer reactions were enhanced with the addition of water to the source. The two modes of ionization provided comparable detection limits for benz[a]anthracene of about 150 pg (signal to noise ratio of 2).

Using a photoionization detector for the off-line optimization of chromatographic conditions, SFC-APCI-MS was applied to the characterization of PAC in complex extracts from coal tar and hydrotreated tar sand oils. Ions corresponding to carbazole (m/z 168) and its alkylated homologues (m/z 182 to 490) were easily identified in a nitrogen-containing PAC fraction of a tar sand oil. Tandem mass spectrometric (MS/MS) experiments performed on the abundant [M+H]⁺ ions of the alkylated carbazoles showed the losses of neutral hydrocarbon fragments from the alkyl side chains.
Figure 1: SFC-APCI-MS background subtracted mass spectra of a test mixture of PAH.
Recent advances in the fabrication of ULSI devices has demonstrated the need for highly purified gases, with impurities at levels below 1 ppb. Consequently, the analysis of impurities in highly purified gases is becoming increasingly important in the semiconductor industry. Normal detectors utilizing GC and GC/MS techniques are not capable of detecting impurities at levels near 1 ppb. We have developed an Atmospheric Pressure Ionization Tandem Mass Spectrometer (API MS/MS) to measure trace impurities in ultrahigh purity nitrogen. This system has the capability of measuring sub-ppb levels of methane, water, oxygen and carboxdioxide in bulk nitrogen. In addition, utilizing the triple quadrupole feature of the analyzer, trace carbon monoxide can also be detected at levels below 1 ppb. This API MS/MS system utilizes a point to plane corona discharge at atmospheric pressure. The source is made of specially treated materials, and is heated, to avoid problems due to the adsorption of impurities on its surface. The triple quadrupole uses 3/4" diameter poles, and patented leaky dielectric endplates for enhanced transmission.

A mass dilution system along with a calibration gas was used to generate the calibration curves. The curves were obtained by monitoring the intensity at the indicated mass to charge ratio. The figures shown below demonstrate the linearity and the sensitivity for detecting the impurities in nitrogen. A linear regression analysis, with 99% confidence bounds, performed on the system response to varying concentrations of oxygen, indicate that the system has a detection limit of 50 ppt for oxygen in nitrogen. Experiments have also been performed on detecting trace impurities in argon, with similar results.
SINGLE-PHOTON IONIZATION MASS SPECTROMETRY WITH A VUV MOLECULAR HYDROGEN LASER SOURCE

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A molecular hydrogen laser is characterized as a photoionization source for mass spectrometry. The laser, shown in Figure 1, is a Blumlein discharge apparatus, with a narrow discharge channel, 330 μm in height, and a low-inductance spark gap switch purged with nitrogen. Since the laser operates in a superradiant mode, there are no optical cavities or mirrors to align. Therefore, the laser is relatively simple to build and easy to operate. Details of the laser construction used in our laboratory have been previously discussed (1,2). The bottom Blumlein plate is wrapped with eight layers of .05" thick mylar dielectric and connected to a high voltage supply operating at 20 kV. With a hydrogen pressure of 45 torr inside the channel, the device outputs 160 nm (7.8 eV) photons from a series of Lyman transitions in the vacuum ultraviolet, with an average peak pulse energy of 30 μJ and a pulse duration of ~1 nsec.

For photoionization mass spectrometry, the laser is coupled to a linear TOF mass spectrometer (Figure 2) through a custom purge tube filled with He. The tube contains two CaF₂ windows sealed with o-rings at the ion source and the discharge channel. Ions are accelerated to 635 eV through a drift tube, 1.5 m in length, which contains a Galileo Electro-Optics Z-stack microchannel plate detector (~10⁸ gain) mounted at the end. Mass spectra are averaged over many laser pulses and stored with a LeCroy 9400A digital oscilloscope, which is linked to an IBM compatible 286 computer via an IEEE-488 interface. An ASYST version 3.0 software package was utilized for data acquisition and analysis. Samples are introduced as solutions through a heated inlet, or as solids using a direct insertion probe.

Types of compounds ionized at or below 7.8 eV include particular classes of molecules such as tertiary and aromatic amines, nitrogen heterocycles, pharmaceuticals, drugs of abuse, and polynuclear aromatic hydrocarbons (PAHs). The laser is a "soft" ionization source which produces only parent molecular ions for most of these compounds. For mixtures of photoactive species, this produces a simplified mass spectrum of only M⁺ ions as demonstrated in Figure 3, for a .5 μl injection of a mixture containing equal volumes of aniline (93), triethylamine (101), N,N-dimethylaniline (121), tripropylamine (143), tri-n-butylamine (185), and trihexylamine (269).

For PAHs, reproducible ion signals are obtained for averages over 500 laser pulses, with detection limits (S/N=3) in the 100 pg range. Selectivity and sensitivity for detection of PAHs in environmental samples is illustrated in Figure 4 for the analysis of a water sample from a drinking fountain spiked at 100 parts-per-trillion with pyrene (202), chrysene (228), benzo[a]pyrene (252), benzo[ghi]perylene (278), and coronene (300). 1 liter water samples of blank water and spiked water were mixed with 120 ml of isopropanol and aspirated through a 3 ml Bakerbond C₁₈ solid-phase extraction column (J.T. Baker, Inc.). Then the columns were eluted with 3 ml of hexane and the residues from the extracts were analyzed directly with an insertion probe on the laser/TOF instrument. Spectra for each sample were recorded by averaging over 200 laser pulses at two different heating times of the probe. While over 14 organic components were identified in a blank water sample extract by GC/MS, the photoionization mass spectra gave clear M⁺ ion signals for PAHs in the blank and spiked samples with a relatively low background from the sample matrix. This is attributed to the fact that the other organic components in the water sample identified by GC/MS have ionization potentials above the 7.8 eV laser threshold.

Selectivity of 7.8 eV photoionization has also been proven for screening of abused drugs spiked in pooled human urine samples. In this case, a simple solid-phase extraction step using a...
Bond Elut Certify column (Analytichem International) was applied, followed by direct analysis of the resulting solid residue with an insertion probe. Urine samples spiked at 200 ppb with cocaine, codeine, morphine, methadone, and phencyclidine yield photoionization mass spectra consisting of intense parent molecular ion signals for the drugs with a few signals from the matrix which do not interfere with identification of the M+ peak.

References
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

ENHANCED SENSITIVITY IN SFC-CIMS OF GLYCOCONJUGATES THROUGH SAMPLE DERIVATIZATION AND FOCAL PLANE DETECTION

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Introduction

The importance of glycosylation in a variety of biopolymers has resulted in a more focused effort to understand carbohydrate structures. A detailed analysis of an oligosaccharide is however, complicated by numerous factors, foremost of which is an isomeric complexity coupled with the general problem in detecting sensitivity. Generally, several times more material is necessary for the detailed structural analysis of a carbohydrate than is the case for other biopolymers.

We have found that SFC-MS can provide an alternative approach to carbohydrate separations and molecular weight detection.1,2 When these materials are conjugated with groups possessing enhanced electron affinity, marked improvement in detection (femtomole) has been observed using negative ion chemical ionization.3 Further structural understanding has been realized through pre-column chemistry and related analyte derivatization.

It is the focus of this study to capitalize on these improvements by capturing this sensitivity and structural detail with a focal plane detector (VG ZAB2-SE-FPD equipped with a plane parallel electric sector and 40% coverage focal plane detector). Preliminary analyses comparing chemical ionization with fast atom bombardment indicated greater freedom for FPD signal amplification and hence smaller sample loading. SFC coupling would provide a similar matrix-free environment for NICI-MS-FPD analysis.

Results

In order to evaluate the relative sensitivities of the point detector (photomultiplier) and FPD, as well as the overall sensitivity of the FPD, a well characterized standard, PFBAB-labelled maltoheptaose, as a peracetylated derivative, was run under identical chromatographic conditions using each detector. Using the photomultiplier, at a resolution of 1000 ppm, scanning from 3000 to 300 amu, 80 femtomoles of PFBAB-maltoheptaose provides a selected ion plot with a signal to noise ratio of 25:1 and spectra with a signal to noise ratio of 50:1. This was commensurate with the initial studies done on a ZAB-SE.3 In previous work, the lower limit of detection was between 10 and 20 femtomoles for this compound.

Figure 1 shows the total ion chromatogram for an injection of 8 femtomoles, using the FPD, at lowest resolution (90°), with the micro-channel plate at 1.25keV and phosphor at 3keV. Signal to noise for this run is approximately 10:1. After peak processing, the spectrum in Figure 2 is obtained. Signal to noise is virtually 100:1.

These data were obtained under conditions where the FPD has not yet been fully optimized, but it is clear that this detector complements the features of SFC-MS well. The low background of SFC-NICIMS and concentration of ion current in the molecular ion are ammenable to the amplification possible with the FPD.

References


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High Sensitivity Detection Effects upon FAB/SIMS Spectra

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High sensitivity detection investigations, originally undertaken to improve sensitivity at high mass, were found to provide significant improvement over the entire mass range. The investigations included increasing the ion impact energy and lowering the impact dynode work function to assure copious desorption/emission of negative particles. Improvement of signal and signal-to-noise has been demonstrated for a variety of ionization modes including EI, CI, Thermospray, FAB/SIMS, Electrospray, Particle Beam, etc. The enhancement of signal is sometimes dramatic, typically greater at high mass, and somewhat similar in effect for the different ionization modes. A few outstanding characteristics have been observed with Electrospray and FAB which will be discussed.

Electrospray spectra contain multiply charged ions which are advantageous in observing high mass sample on a limited range mass spectrometer. In addition, improvement in sensitivity for the multiply charged ions using the High Energy Dynode (HED) detector is greater than that for singly charged ions, revealing effects of impact ion mass upon signal enhancement.

The background noise typically observed in FAB/SIMS spectra measured on quadrupole mass spectrometers varies with the HED voltage differently than signal. This is probably due to the particular mix of particles desorbed during the ion (atom) surface bombardment. During the desorption process sample ions, liquid matrix ions and scattered primary ions/atoms are produced in the ion beam and focused toward the analyzer. The sample and its fragments along with matrix molecular ions make up the readable spectrum. The noise is composed of chemical noise (matrix fragments) which appears at each amu on the mass scale and scattered ions which are not mass analyzed. Chemical noise is generated by the desorption/ionization process and appears to the detector as signal. However, scattered ions having a higher energy than the others display characteristics that can be observed at the detector.

The tests reported in this abstract were performed on an HP5982 outfitted with a Cesium Ion Gun or a DIP Gun to produce the ions and an HED Detector used to increase detection sensitivity. Spectral signal was measured in the selected ion mode while the voltage to the dynode was varied between -8kV and +8kV. The general characteristics of molecular ion signal variation with HED voltage using gas and Cesium guns are similar to those reported in past HED studies (Ref. 1). However the non-mass analyzed background noise variation is distinctly different from the mass analyzed spectral signal (including sample, matrix, and chemical noise). Figure 1 shows the signal variation with voltage from +7kV to -8kV for the cesium ion peak. The minimum observed near -2kV and the maximum near -6kV have been observed many times previously. The structure in the vicinity of zero appears to be dependent upon the ion trajectory and the impact region within the horn of the CDEM (Ref. 2). A similar minimum occurs near -2kV in Fig. 2, which shows the variation of noise with HED voltage. In this case the maximum is a much sharper peak near -3kV with a steeper decline above this voltage. This region is not as well understood as is the region to the left of -2kV, which appears to relate to the structure near zero voltage in Fig. 1, while its spread is attributed to the higher energies and energy spread of the scattered ions generating the noise.

Figure 3 illustrates the exceedingly favorable effects of using HED voltage for moderately high mass FAB spectra. The signal increases, the noise decreases and both the signal and signal-to-noise increase by over two orders of magnitude.

Fig. 1. Measurement of Cesium ion (133 amu) abundance variation with HED voltage produced by Cesium ion bombardment.

Fig. 2. Noise variation with HED voltage during Cesium ion bombardment.

Fig. 3. Comparison of FAB spectra of Aerosurf with and without HED voltage showing a signal gain and signal-to-noise ratio gain of about two orders of magnitude.
The value of post mass filter acceleration devices in improving sensitivity has been demonstrated by Daley, Dietz, and others (1,2,3). These devices work by increasing the velocities of ions as they leave the mass filter, which increases the conversion of ions into electrons at the detector. Since all ions in a quadrupole mass spectrometer have similar energies (mv^2/2) and conversion of ions to electrons at the electron multiplier depends on velocity (v), higher mass ions provide lower signals with a standard electron multiplier and benefit more from the acceleration provided by a high energy dynode detector (HED).

Diagrams of an HED multiplier and a standard (non-HED) multiplier are shown in Figures 1 and 2, respectively. In the normal mode of operation, the high energy dynode is operated at 4-8 kV to accelerate ions leaving the quadrupole and to convert them into electrons, which are then accelerated into the horn of the multiplier. The dynode can also be operated at low voltage (0-255 V) to focus the ions directly into the horn of the multiplier. In the low voltage mode, the dynode resembles an "X-ray" lens and the HED shows sensitivities comparable to that of a standard multiplier.

The sensitivity improvement provided by the HED was determined by analyzing fourteen compounds with the HED operated in the standard, high-voltage mode, vs. with it operated in the low-voltage, "X-ray" mode. These studies were done with an HP5989A GC/LC/MS, operated in the El, Cl, particle beam (PB), and thermospray (TS) modes.

The HED produced sensitivity improvements of two to sixteen times in the positive ion mode, with the degree of enhancement increasing with molecular weight. In general, dividing the molecular weight by 100 provided a conservative estimate of the signal-to-noise enhancement provided by using the dynode at high voltage. For example, the HED improved sensitivity for 10 pg hexachlorobenzene (mass 284) by a factor of three, but improved sensitivity for hexaphenylbenzene (mass 534) by a factor of six. (See Figure 3.) The overall results are summarized in Table 1. Since the sensitivity gains were proportional to molecular weight, the HED proved especially beneficial for the LC/MS analyses of higher molecular weight compounds.

Table 1. HED Sensitivity Enhancement Increased with Molecular Weight

<table>
<thead>
<tr>
<th>Weight Range (amu)</th>
<th>Enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>100-300</td>
<td>2-4 X</td>
</tr>
<tr>
<td>300-500</td>
<td>3-6 X</td>
</tr>
<tr>
<td>500-600</td>
<td>6-16 X</td>
</tr>
</tbody>
</table>

Figure 1. High Energy Dynode Detector

Figure 2. Standard Detector

Figure 3. HED Increased Sensitivity 6X at Mass 534.
The growing popularity of very small and relatively low cost instrumentation for both residual gas analysis (RGA) and gas chromatography/mass spectrometry (GC/MS) has focussed attention on every area of the instrument. These seemingly unrelated techniques have a number of systems in common - one of the most important of which is the detector. Standard single channel electron multipliers (CEMs) are commonly used because of their excellent performance characteristics and stability even after repeated cycling between vacuum and atmosphere. The major thrust in recent years has been on reduced size and cost with little or no sacrifice in performance. Many CEMs suffer from the fact that they are relatively large devices (up to three inches long), and in some cases offer more performance than is really necessary (e.g. most RGA applications have little need for an analog gain of up to $10^7$ or more).

We have developed a compact ion detector based on a microchannel plate (MCP) which reduces the length of a typical detector by a factor of three while at the same time retaining many of the performance features of single channel detectors and offering the potential for even higher dynamic range than standard single channel devices. For higher gains required in some applications, a second MCP may be added.

Two variations of the detector are possible as shown in the figure below. The first is a device in which the MCP is located off-axis in order to reduce noise due to photons and neutrals emitted from the quadrupole. This detector would be operated in a manner similar to standard CEM detectors.

The second detector is based on an MCP with a 6mm center hole which allows an undeflected ion beam to pass through the detector. This undeflected beam may be collected in a faraday cup connected directly to an electrometer amplifier. For very small signals, the beam is deflected onto the MCP for amplification of $10^4$ to $10^7$ depending on the configuration. The deflection is accomplished by applying a high voltage to selectively electroded areas of the MCP detector. This has the potential advantage of allowing two or more detectors to be fabricated in a single housing. Initial experiments indicate that good sensitivity is obtained if the MCP voltage is kept high enough to accelerate the ions sufficiently into the detector. The device utilizes a common anode/faraday cup configuration for simplicity and cost savings.

Preliminary characterization data for both types of detector will be presented as well as recommendations for further improvements.
Direct Thermal Analysis of Materials Utilizing the Short Path Thermal Desorption System

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This new technique permits the analysis of solid samples without the prior need for solvent extraction or other sample preparation. A variety of types of materials were analyzed including Food Products, Packaging Materials, Finished Manufactured Samples, Pharmaceuticals and Forensic Samples. The solid sample to be analyzed is inserted directly into the Glass Lined Stainless Steel (GLT) Thermal Desorption Tube. The new "Short Path Thermal Desorption System" was utilized to permit the direct sparging of the volatile and semi-volatile components from the solid samples directly into the GC injection port with subsequent GC and MS analysis. This technique permits the maximum sensitivity of analysis due to this direct injection technique.

The Scientific Instrument Services "Short Path Thermal Desorption System" was attached to the injection port of a H.P. 5971 MSD. A J&W DB-5, .25 mm x 25 meter, 0.25 u film thickness capillary column was used for this study. The GC was used in the splitless mode at a flow rate of 0.5 ml/min.

Desorption Tube for Direct Thermal Analysis

Experimental

One to 3 mg samples of the various solid samples to be analyzed were inserted into the GLT tubes between two quartz wool plugs. These tubes were then attached to the Short Path Thermal Desorption System and fitted with a syringe needle. The sample was flushed with helium carrier gas and then autoinjected into the GC injection port. The heating blocks were heated to the sparging temperature indicated and closed around the desorption tube to ballistically heat the sample to the set temperature. This combination of heat and flow sparge any volatiles or semi-volatiles into the GC injection port were they are subsequently trapped in a narrow band at the front of the GC column which has been cryo-cooled to -40° C. The sample is collected for 10 minutes after which the GC is programmed at 10° per minute up to 280° C, to elute the products which are subsequently analyzed via the Mass Spectrometer.
Results and Discussion

Food Products. Dried vegetative materials which are relatively low in moisture content can be analyzed via this technique. A wide variety of spices have been analyzed and the various terpenes, hydroxy-terpenes and sesquiterpenes identified. Samples containing high oil content such as peanuts can be analyzed by this technique instead techniques such as soxhlet extractions. Flavored candy samples can be analyzed directly to identify the artificial flavors and other additives.

Packaging Materials. Plastic food wraps, tapes, papers, cardboard, packaging containers, and other packaging products can be analyzed via this technique to identify the volatile components present that may be leached out in food products. With the increased use of recycled papers and with the increased concern over the leaching of chemicals, inks, preservatives and insecticides into food products, this technique is extremely versatile in identifying these materials.

Manufactured Products. Virtually any solid material can be analyzed via this technique. Samples analyzed include powdered laundry detergents, synthetic fibers, nylon rugs, wood products, plastics, waxes, and other finished manufactured products.

Forensic Materials. The direct thermal analysis technique lends itself to many areas of forensic analysis. Pharmaceutical samples can be analyzed to determine the residual solvents present. Materials from the scene of suspected arson crime scenes can be analyzed for accelerants.

Conclusion

The technique of direct thermal analysis utilizing the Short Path Thermal Desorption System has proven to be a valuable and versatile technique for the analysis of a wide variety of solid type samples. Due to the direct injection technique, the maximum sensitivity of analysis is possible and the labor time for sample preparation is minimized.
Improved Precision of Mass Spectral Response by Digital Control of Ion Energy and Mass Resolution

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Most GC/MS methods promulgated by the U.S. Environmental Protection Agency (EPA) require that the mass spectrometer be tuned in a specific manner. In particular, these methods require that the mass spectrometer be tuned to generate spectra of 4-bromofluorobenzene (BFB) and decafluorotriphenylphosphine (DFTPP) which meet specific relative intensity and resolution criteria. This may be a difficult and time-consuming task, requiring that the mass spectrometer be detuned from its optimal performance conditions. It is strongly desirable to avoid such time-consuming tasks in a production environment, so sample throughput can be maximized.

This paper describes a simple method of tuning the mass spectral response of a benchtop quadrupole mass spectrometer. It also demonstrates its applicability for satisfying EPA tuning protocols.

INTRODUCTION. In a quadrupole mass spectrometer the magnitude of the DC and RF voltages determines the mass and, in theory, a constant DC/RF ratio defines the resolution. A DC/RF ratio changing as a function of RF is often used for better control of mass resolution and intensity across the mass range. This is frequently achieved in hardware by increasing the DC by some fraction of the RF:

$$[DC] = [DC] + k[RF]$$

where $k$ is varied as a tuning parameter. Altering the $k$ parameter affects all masses in the spectrum.

The potential between the ion volume and the entrance aperture to the quadrupole rods, known as the ion energy, also exerts a strong influence on the spectrum. Increasing ion energy with mass is often applied to improve ion transmission from the ion volume to the quadrupole. This is usually performed linearly with mass:

$$E = a + b[RF]$$

where $a$ and $b$ are constants.

Together, the lens voltages, ion energy, and the DC/RF ratios define the observed relative ion intensities across the mass spectrum. The effects of the lens voltages interact, and adjusting one generally necessitates adjustment of others. This makes tuning a quadrupole mass spectrometer to match BFB or DFTPP somewhat problematic.

This paper demonstrates a method of defining the ion energy and DC/RF ratio at up to ten masses across the spectrum, and then ramping them in a piecewise linear manner. No changes to lens potentials are required. This allows the relative spectral intensity to be defined locally, with minimal effects elsewhere in the mass range. This technique, called OptiTune™ (Trademark of the Perkin-Elmer Corporation), allows the facile matching of reference spectra criteria.

EXPERIMENTAL. The ion optics were first tuned for optimal sensitivity across the mass range. The reference spectrum was then matched by DC/RF and ion energy adjustments alone.

These adjustments were made by observing the spectral intensities produced by a controlled leak of perfluorotributylamine (also known as PFTBA and FC-43) at ten of its characteristic masses (19, 50, 69, 131, 219, 264, 314, 414, 502, and 614). The ion energy and DC/RF ratio at each these masses were then modified.
so that DFTPP or BFB would yield a spectrum meeting EPA criteria upon injection. Once an acceptable DFTPP or BFB spectrum was obtained, the PFTBA spectrum was recorded.

EPA criteria may also be achieved by tuning the mass spectrometer to precisely replicate the recorded PFTBA spectrum.

RESULTS. DFTPP was chosen to demonstrate the effects of altering the DC/RF ratio at one mass of PFTBA.

Figure 1 shows the results of decreasing the DC/RF ratio and increasing the observed intensity of DFTPP's mass 275. Note that the spectrum has been simplified, by removing some masses (for heuristic purposes). Figure 2 displays the reverse, with a greatly reduced mass 275 intensity.

DISCUSSION. The optimal tuning of the mass spectrometer can usually generate a spectrum close to meeting the EPA criteria. Only small changes of relative intensity, typically less than 10 or 20 percent, are required for complete compliance. The displayed spectra in exhibit much larger variations in relative intensity for demonstration purposes. Smaller and more typical changes in intensity would have correspondingly smaller effects on the intensities of nearby masses.

There are two significant advantages to tuning by piecewise linear changes of the ion energy and DC/RF ratios.

First, the intensity response across the mass range can be adjusted locally, over a narrow mass window, without perturbing those mass intensities which may already meet the EPA criteria. This significantly reduces the need for iteration between tuning adjustment and injection of standards, thus saving time.

Second, the mass spectrometer operates at optimum voltages on its ion optics. Small drifts in voltage have minimal effect. In a conventional approach, the mass spectrometer would be de-tuned to meet the EPA criteria. Rather than operating at a maximum or minimum optimum for a particular voltage, the mass spectrometer operates where the response to a particular voltage may be sharply sloping. Small drifts in voltage now have significant effect.

CONCLUSIONS. This paper has demonstrated a novel approach to tuning a mass spectrometer to meet EPA criteria. The piece-wise linear fit of ion energy and DC/RF ratios allows:

- precise control of mass spectral response
- minimal interaction across the mass range
- much more flexibility than the conventional approach of varying only the slope of the DC/RF operating line or ion energy
- intrinsically more valid spectra than a software normalization based on PFTBA response.

Figure 1. Increased Mass 275

Figure 2. Decreased Mass 275
MODIFICATION OF A QUADRUPOLE MASS SPECTROMETER FOR LIQUID SIMS

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The advent and refinement of liquid SIMS during the past decade offers the possibility for truly routine mass spectrometric analysis of polar, thermally-labile biomolecules. These types of compounds are not readily analyzed using classical ionization methods such as electron ionization and chemical ionization. Many older mass spectrometers were not designed to accommodate the liquid SIMS experiment. In this report, we have reconfigured one such instrument, a Hewlett-Packard HP5985A GC-MS, to perform liquid SIMS.

An important consideration in adapting the instrument was to keep the modifications relatively simple, such that any moderately equipped machine shop would be able to effectuate the changes. The modifications consisted of first welding a vacuum flange to the source-housing manifold to accommodate a commercially available focusing cesium ion gun (Antek model Cs-160-250B, Antek, Palo Alto, CA). Afterwards, a new secondary ion source block and secondary ion collection lens assembly were constructed. A sample probe with detachable tip was also fabricated.

Each constructed item was taken through an independent optimization procedure. The height of the added flange was positioned such that a minimum clearance (<1/4 inch) existed between the final lens of the cesium ion gun and the magnet yoke of the conventional EI/CI source. In this way, the mass spectrometer could be operated in the normal EI and CI modes without removing the cesium ion gun. The diameter of the added 'receiver' flange is 3/8 inch larger than the diameter of the ion gun mounting; this allows for manual centering of the primary ion beam.

Optimization of the aperture width, and placement of the added secondary ion collection lens has also been performed. Figure 1 shows the 3-dimensional plot of signal counts at peak maximum vs. lens distance from the probe tip vs. lens diameter. Four different lens distances and four lens diameters were evaluated. A 9 mm diameter lens placed at 6 mm from the target was shown to yield the greatest secondary ion signal. The target reservoir diameter was also empirically optimized; a 3 mm diameter resulted in the best secondary ion signal, as shown in Figure 2.

A conversion dynode detector (Phrasor HED, Phrasor Scientific, Duarte, CA) was purchased to replace the conventional electron multiplier detector in order to improve sensitivity especially at the higher mass end of the quadrupole analyzer (upper mass limit = m/z 1000).

The new capabilities for liquid SIMS have been demonstrated for various peptides, acyl carnitines, nucleosides, and glycoconjugates. Displayed in Figure 3 is the mass spectrum obtained for the peptide proenkephalin (Tyr-Gly-Gly-Pho-Met-Arg-Gly-Leu).

![Figure 2: Signal counts at peak maximum vs. probe tip reservoir diameter for four different probe tips.](image)
Figure 1: 3-Dimensional plot of signal counts at peak maximum vs. lens distance from probe tip vs. lens diameter.

Figure 3: Liquid SIMS spectrum of the peptide proenkephalin.
LASER PYROLYSIS GC/MS OF SINGLE COAL AND COAL MODEL PARTICLES

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Laser pyrolysis GC/MS using an electronically pulsed cw CO₂ laser focussed at the center of a Paul trap type particle levitation device connected to a Paul trap type spectrometer (Finnigan MAT ITMS) by means of a short, ballistically heated capillary GC column (see Figure 1a) has proved to be a valuable technique for studying the devolatilization (pyrolysis + desorption) behavior of single, 100-150 μm sized coal particles at very high heating rates (10⁵-10⁶ K/s) [1,2].

A major advantage of the Paul trap type particle levitator, better known as EDB (Electro Dynamic Balance) is the absence of hot, adsorptive and/or catalytically active surfaces in the reaction zone. However, during the course of our experiments we encountered several serious disadvantages including difficulty in stabilizing irregularly shaped coal particles which causes the loss of many laboriously prepared and selected coal particles and introduces further problems when trying to obtain reliable optical images of single coal particles, e.g., for the purpose of temperature measurements. A second complication, encountered in trying to optimize experimental conditions, is the notoriously high interparticle heterogeneity in coals which is compounded by the unavailability of well defined coal model particles.

These experiences prompted us to design a modified experimental set-up, shown in Figure 1b, in which the EDB cell is replaced with a simple electron microscopy (EM) grid (400 mesh, 78% open) to support and stabilize particles while providing markedly improved collection efficiency for laser pyrolysis and desorption products, as illustrated in Figure 2. Comparison of the two GC/MS profiles in Figure 2 shows a high degree of qualitative similarity while illustrating the higher yield and improved signal to noise ratio of the EM grid technique. The improved signal quality is especially important for detecting and identifying minor components, e.g., reflecting subtle changes in pyrolysis mechanisms as a result of the high laser heating rate. Thus far, we have not detected any effect of the metal (copper; gold coated versions available) grid on the yield and composition of devolatilization products.

The need for coal devolatilization model particles of known composition provided the incentive to impregnate 100-150 μm sized Spherocarb particles (widely used for modeling coal char combustion behavior) with known quantities of coal-derived bitumen as well as soluble polymeric model substance, including fossil resin from Wasatch Plateau field (Utah) coal as well as recent Aspen lignin [3]. Preliminary results shown in Figures 3 and 4 illustrate the feasibility of this approach since characteristic pyrolysis products of resin and lignin are readily detected and identified among the devolatilization products from single, impregnated Spherocarb particles. Unfortunately, recent work by Sarofim and co-workers [4] has cast doubt on the presumptive homogeneity of Spherocarb particles with respect to density. This may limit the usefulness of impregnated Spherocarb particles for the development of reliable devolatilization standards in view of the potentially wide variation in absorptive and desorptive properties.

ACKNOWLEDGEMENTS
This work was sponsored by the Advanced Combustion Engineering Research Center. Funds for this center are received from the National Science Foundation, the State of Utah, 23 industrial participants and the U.S. Department of Energy.

REFERENCES
Figure 1. Schematic diagram of CO$_2$ laser pyrolysis TLGC/MS system consisting of a pulsed CO$_2$ laser, an electrodynamic balance ("particle levitator"), a transfer line GC column and an ion trap mass spectrometer. (b) Laser Py-GC/MS configurations using electron microscope grid with crossed laser beams. Note special sample inlet and capillary GC transfer line connection to ion trap mass spectrometer (ITMS).

Figure 2. Comparison, verification and peak identification of EM grid and EDB levitated CO$_2$ laser pyrolysis GC/MS of Illinois #6 coal.

Figure 3. Selected ion chromatograms obtained by laser Py-GC/MS analysis of a single, lignin impregnated Sphero Carb particle. Note presence of characteristic hardwood lignin building blocks.

Figure 4. Laser desorption GC/MS profiles of a single Sphero Carb particle impregnated with fossil resin from Wasatch Plateau field (Utah). Note characteristic sesquiterpenoid building blocks.
THE DETERMINATION OF STABLE ISOTOPE INCORPORATION BY GC-FI/MS

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INTRODUCTION

Combined gas chromatography-field ionization mass spectrometry (GC-FI/MS) has proven to be a useful technique in this laboratory for the determination of stable isotope incorporation in the study of mechanistic organic chemistry. The endocyclic restriction test is being developed here to determine the allowed reaction geometries for substitutions at various heteroatoms. This work provides the only experimental approach to the determination of reaction trajectories at non-stereogenic atoms. In order to determine if a reaction is intra- or intermolecular, double-labeling reaction experiments are employed with measurement of the isotopic distributions in the reactants and products by GC-FI/MS. This technique is extremely useful since conventional isotope ratio measurement techniques provide limited information when dealing with mixtures of small amounts of material. The electron ionization (El) spectra of many compounds examined posses a large (M-H)+ peak which interferes with H/D incorporation calculations.

HARDWARE AND METHODS

The GC-FI/MS isotopic incorporation measurements were performed using a VG 70-VSE mass spectrometer (VG Analytical, Ltd., Manchester, U.K.) employing a VG retractable, high temperature GC capillary column re-entrant and the new-style VG FI/FD ion source. The re-entrant is mounted in a bellows mechanism with a scissors jack motion device. This permits the end of the capillary GC column to be brought into direct proximity (within 1-2 mm) of the emitter wire in the FI/FD ion source. The re-entrant can be heated to over 400° for high boiling compounds. The re-entrant is completely retractable to allow the ion source to be installed and is adjustable in three dimensions for the optimal positioning of the end of the GC column with the emitter wire. The new-style FI/FD ion source is of an open design and does not have a source block which minimizes memory effects and background problems. The extraction focusing element consists of two parallel metal tubes which are internally heated. The small surface area of the tubes combined with the heating minimize condensation and surface reaction problems common to other type FI/FD ion source designs. Data were collected using electrical voltage scans over a narrow window around the molecular ion region.

EXPERIMENTAL RESULTS

The precision of the technique was measured by performing five consecutive GC-FI/MS runs of a mixture of equal amounts of N-isopropyl-3-phenylthiopropanecarboxamide and N-isopropyl-4-phenylthiobutanecarboxamide with 250 ng of each injected on the GC column. These are compounds whose product reactions have been examined with the aide of GC-FI/MS. The data indicate that the technique is reproducible to better than +/- 5%.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R.A. (M+1)+</th>
<th>R.A. (M+2)+</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-isopropyl-3-phenylthiopropanecarboxamide MW = 223</td>
<td>14.24 ± 0.22 (1.54%)</td>
<td>5.46 ± 0.13 (2.38%)</td>
</tr>
<tr>
<td>N-isopropyl-4-phenylthiobutanecarboxamide MW = 237</td>
<td>15.35 ± 0.37 (2.41%)</td>
<td>5.75 ± 0.15 (2.61%)</td>
</tr>
</tbody>
</table>

The reaction trajectory of the substitution of iodine by bromine in 3'(o-Bromophenyl)propyl-11-iodoundecanyl ether (Figure 1) has been examined employing double labeling experiments. Reactions in t-BuLi with subsequent reduction by MeOH were performed using the compound with natural abundance H and Br and with a 1:1 mixture of enriched compounds (o-Br and o-Br'/I'-D2). The GC-FI/MS data...
(Figures 2-3) indicate that the Br substitution is intermolecular and endocyclic because the products retain the label within the molecules with no evidence of products resulting from the intramolecular transfer of Br.

1) l-BuLi
2) MoOH

FIGURE 1. REACTION SCHEME

FIGURE 2. F/MS OF NATURAL ABUNDANCE COMPOUNDS

FIGURE 3. F/MS OF ENRICHED COMPOUNDS

CONCLUSIONS

GC-FI/MS is an extremely useful technique for the determination of stable isotope incorporation applied to the study of reaction trajectories and kinetics. The technique is ideally suited to measuring incorporation in complex mixtures. GC-FI/MS is quite sensitive, as little as 10-50 nanograms of some compounds are needed. The technique provides measurements reproducible to better than +/- 5%, it is easy to perform, and does not suffer from the interferences present in EI since FI usually produces mainly molecular ions.

ACKNOWLEDGEMENT

The 70-VSE mass spectrometer was purchased in part with a grant from the Division of Research Resources, National Institutes of Health (RR 04648). The reaction trajectories research was funded by a grant to P. Beak from the National Science Foundation (CHE-875607).

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An Automated Capillary GC Column Backflush System for Rapid GC/MS Analyses

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A current trend in GC/MS analysis is to achieve the detection and quantitation of large numbers of analytes in a single analysis in order to reduce overall costs. Considerable effort is also being expended on developing methods to analyze compounds having a wide variety of functional groups in a single analysis. The removal of all interfering compounds during sample cleanup procedures is not always possible when these encompass a wide variety of functional groups. Although high resolution mass spectrometry has been used to eliminate interferences arising from coeluting compounds which yield ions that can be mass resolved, this still does not obviate the need to remove residues which may result in degradation of column separation efficiency. In addition, the less-selective cleanup procedures and the long capillary GC columns which are typically used to achieve adequate resolution of many chemical components in such analyses often make it necessary to bake off the capillary column for extended periods of time after an analysis.

The objectives of the present work were to reduce or eliminate GC column bake-off time, to increase the time intervals between MS source cleanings, and to prolong the life of the GC capillary column by avoiding deposition onto the column of non-chromatographable residues.

Although the idea of using a capillary GC pre-column, and backflushing compounds having retention times greater than the analytes of interest, is well known, commercial systems which employ these methods are not readily available. Figure 1 shows a block diagram of an automated backflushing system designed at our laboratory. We have successfully employed this design to reduce turn-round time for a number of types of analyses on three different GC/MS systems. A backflush tee of the usual design is employed with this system.

Several advantages result from the use of this backflushing system. This system allows the removal of all peaks eluting after the analytes of interest, which can significantly decrease column bake-off time between analyses. Retention time characteristics of the analytical column are preserved since the pre-column prevents degradation of the primary analytical column by trapping non-chromatographable residues. This pre-column can be easily and inexpensively replaced. In cases where the capillary column is directly connected to the mass spectrometer, the intervals between required source cleanings are usually extended, since the late-eluting higher boiling compounds do not enter the source.

The backflush system described here has two limitations. First, installation of such a system requires replumbing the gas chromatograph during installation, which may void the manufacturer's warranty. Another possible problem concerns the column connection to the backflush tee. Since the connection between the pre-column and the analytical column cannot be a gas-tight connection, the solvent front from the injection can partially diffuse into the dead volume of the backflush tee connection. When redirection of the carrier supply occurs at the backflush time, a portion of the injection solvent that diffused into the dead volume will be redirected onto the analytical column. Under certain conditions, this re-introduced solvent peak can coelute with an analyte and represent an interference. However, a slight adjustment of the actual time of backflush will alter the elution time of the reintroduced solvent peak, and result in separating it from the analyte peak of interest.

The backflush system described here has been in continuous use on several GC/MS systems at our laboratory for a period of three years, and has functioned successfully in all cases.
Figure 1. Block Diagram of Automated Capillary GC Column Backflush System
Implementation of Advanced GC/MS Peak-Detection Algorithm

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In the past, detection of chromatographic peaks in a GC/MS system has generally relied on a set of simple rules which delimit the peaks. Problems that frequently arise from use of such simple rules include incorrectly drawn baselines; the beginning or end of a delimited peak may either be on the side of the peak or in the tail far from the peak center; drop markers which separate closely eluting peaks may either be missing or are arbitrarily inserted; and finally, peaks may be found in the background noise. Such problems are most severe with low signal-to-noise peaks and late-eluting peaks that are very wide. These problems result in inaccurate quantitation, and possibly, misidentification of the analytes in a GC/MS run.

The objective of the present work was to create a new rule-based GC/MS peak detection algorithm that would yield accurate, consistent quantitation of analytes in the GC/MS analyses of complex environmental samples. It was intended that this algorithm would also give accurate signal-to-noise values for each analyte delimited, and provide detection limits when the analyte was not found.

The peak detection algorithm processes the data (TIC, single ion, and so on) three separate times. The first pass determines the background noise, the second pass determines the baseline points, and the third pass finds all the peaks. Other data processing may also occur, such as Fourier smoothing, that yields better peak centroiding and more accurate separation of closely-eluting peaks. The present algorithm gives the analyst the option of setting variables that aid the peak detection algorithm. These variables are peak width, background noise, and a parameter for setting baselines. If no peak width is specified, the algorithm defaults to a peak width of five scans. Although the algorithm works well with this default value, it works even better if the actual peak width for an analyte is specified. The actual peak width is easily determined from a standard injection.

The determination of background noise is integral to achieving good peak delimitation with the algorithm. The background noise is found by looking at all the acquired data around the region of quantitation and determining the regions in which no peaks appear, and averaging those regions to determine an average background noise level. For TIC this noise level changes as run time increases. The analyst may set this to some default value if so desired, which is generally the case for full scan work, when the noise baseline for most masses is screened out by the GC/MS data acquisition. This part of the algorithm uses more than 50 rules.

After the background noise is determined, all the baseline points are predetermined before any actual peaks are delimited. The algorithm finds the baseline points in the regions that were determined to have no peaks present. The frequency cannot be greater than the peak width. A baseline point TIC value is close to the middle TIC for the background noise in the region in which it is set. The algorithm defaults to drawing relatively flat baselines. The analyst has control over the rate of change and number of baseline points, and may change these all the way up to a valley-to-valley baseline (skim) for all delimited peaks. This part of the algorithm uses more than 75 rules. At this point, the actual peak detection may be accomplished.

The algorithm searches for possible peaks on a scan by scan basis. When a possible peak is found, the starting and ending scans may be adjusted if chromatographic distortions are detected. The peak must then pass many requirements that include minimum height (signal-to-noise), minimum area, minimum peak width, and so on. If it passes all of the requirements, a peak packet is stored and the algorithm then searches for
another peak. This part of the algorithm uses more than 150 rules.

The rules for determining a peak include very few special-case rules that apply only to very specific sets of conditions. Most of the rules apply to the majority of the delimited peaks. A sample set of rules governing the determination of a drop marker is given below:

```java
if ( TIC apex - TIC start > 2 * minimum height ) |
if ( TIC present - TIC end >= 2* Minimum height ) |
   TIC next - TIC end >= 2 * minimum height ) |
if ( TIC present + minimum width / 4 > TIC end ) |
   if ( apex - start ) minimum width / 4 &&
      end - apex > minimum width / 4 )|
   if ( previous peak not dropped &&
      end - start >= minimum width ) |
      ( previous peak dropped &&
      end - start >= minimum width / 2 ) ) |
if ( TIC end > TIC end + 1 .. TIC end + minimum width / 4 ) |
   drop marker is inserted
```

As shown in this example, the minimum height, as determined by the noise and the width of the peak, are the only considerations. This is extremely important in the functioning of the algorithm.

This new algorithm has been successfully implemented in the MACH 3 software system of KRATOS Analytical. Additional refinements to the present set of rules will be made to further improve the ruggedness of the algorithm.
A Computer-Controlled Pressure Regulated Sample Inlet System
For Fourier Transform Mass Spectrometry

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Fourier transform mass spectrometers are routinely used for ion-molecule reaction studies. They are usually equipped with leak valves for introducing gas phase samples at constant pressure. The sample inlet system should be continuously adjustable over an FTMS operating pressure range of $10^{-3}$ to $10^4$ torr. The sample pressure should be stable at the desired pressure for at least a few minutes for routine FTMS experiments. For two-dimensional FTMS experiments, the sample pressure has to be stable for several hours. In this work, a feed-back pressure control device was designed and constructed to achieve constant pressure over a long time period. As shown in Figure 1, a single board microcomputer is used to read the pressure from an ion pump and to drive a leak valve through a stepper motor. An assembly code program of the microcomputer was written to allow the operator to input the desired pressure, pressure tolerance, and response time. Once a START command is given, the device automatically increases (or decreases) to the desired pressure value in about five minutes. A stable pressure (within 5% over a 3 hour period) is maintained by the gas sample inlet system.

The algorithm for operation of the device is outlined in Figure 2. First, the ion pump current-to-pressure calibration table is loaded and several control parameters are specified by the user. These include the desired pressure ($P_d$), the pressure tolerance ($DP$), and the response time ($T$). $P_d$ is the pressure under which the experiment is to be conducted. $DP$ is the acceptable pressure range for the experiment. $T$ is the response time for the pressure change with the leak valve turning, a characteristic which has to be determined experimentally. For the configuration of our instrument, it is several seconds. After the parameters are set, the computer reads the current pressure ($P$) and compares it with $DP$. If $P - P_d < -DP$, the stepper motor turns the shaft of the valve 7.2° in the valve-open direction. If $P - P_d > DP$, the stepper motor turns the shaft the same amount in the valve-close direction. Otherwise, no signal is issued to turn the stepper motor. The controller uses this procedure to maintain the pressure between $P_d - DP$ and $P_d + DP$.

This device has been installed on our lab-built FTMS instrument for running two-dimensional FTMS experiments. This design provides a starting point for future automation of FT mass spectrometers.
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**Fig. 1** Block Diagram of the Computer-Controlled Pressure Regulating Device

**Fig. 2** A Flow Chart for the Algorithm for the Pressure Control Device
INVESTIGATION OF A UNIQUE CHEMICAL BACKGROUND DERIVED FROM A CERAMIC PROBE TIP ASSEMBLY

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Introduction
An investigation was made into the identity, origin, and removal of a unique chemical background originating from the ceramic probe tip assembly of the VG 70 series mass spectrometer, observed under El conditions. The background was quite intense at ambient temperature, and significantly interfered with the analysis of unknowns at nanogram quantities. Subsequent shipments of the probe tip assemblies showed the same background ions. The background could be eliminated by heating the probe assembly in the vacuum lock, but was re-introduced upon brief (seconds) exposure to the laboratory atmosphere. A series of experiments were performed to understand and eliminate this problem.

Experimental
A VG 70VSEQ mass spectrometer was used to obtain El spectra of the untreated probe assembly and of the probe assembly after exposure to D2O and methanol. El spectra of boron oxide (Aldrich) were obtained by placing the sample neat in an alternative probe assembly consisting of a stainless steel base and ceramic tip. Methanol extracts of the boron nitride probe assembly base and boron oxide were analyzed using El and ammonia desorption chemical ionization (DCI). An Electroscan E20 electron microscope equipped with a Princeton Gamma-Tech Sili X-ray detector was used to obtain X-ray emission patterns and micrographs from two probe assembly bases. One probe base was untreated; the other had been sonicated in methanol for 30 minutes. Micrographs were obtained with the high resolution environmental secondary electron detector (ESD). X-ray diffraction patterns of boron nitride (Aldrich), boron oxide (Aldrich), and powdered probe assembly base were obtained using a Scintag XDS 2000 X-ray diffractometer with an 1800W copper X-ray source and an Ortec high purity germanium detector.

Results
The composition of ions in the background spectrum was determined using high resolution El mass spectrometry, and was found to contain boron, hydrogen, and oxygen. The base of the ceramic probe tip assembly used is made of boron nitride (BN) and was the source of the contamination.

Further investigation showed that the background spectrum could be altered by exposure to D2O and methanol. Application of a few drops of D2O to the probe tip assembly shifted peaks by 2 and 3 amu, as predicted by the proposed structures of fragment ions. Similar exchange of the active protons with methanol was demonstrated. This suggests that accidental exposure of the probe assembly to a protic solvent or other reactive species might introduce artifacts into a sample spectrum. Cleaning the probe assembly base by sonicating in methanol effectively removed the background but left the probe base extremely brittle and, therefore, unusable. A comparison was made between the probe assembly background and boron oxide, examining ambient temperature El spectra as well as El and ammonia DCI spectra of methanol extracts. Spectra from the two sources were similar, suggesting similarities in composition.

Electron scanning electron microscopy (ESEM) and energy dispersive X-ray (EDX) analysis were used to compare the surfaces of an untreated sample probe base and a base which had been sonicated in methanol. The X-ray emission spectra show that surface oxygen, chlorine, potassium, silicon, and carbon found in the untreated probe are removed during the cleaning step, leaving boron and nitrogen only. Cleaning with methanol removes the surface and exposes the internal crystal structure, shown by the micrographs.

X-ray diffraction patterns were obtained on a powdered BN probe base (untreated) and on boron nitride and boron oxide standards, to study the bulk material. The pattern for the probe base is identical to that of the boron nitride standard, and does not contain evidence that boron oxide may be present in the bulk material. This suggests that the background is primarily a surface phenomenon.

A solution was found in alternative materials. Two options which have been successfully used are a stainless steel probe assembly base (with ceramic tip) and a one-piece quartz probe tip assembly.
El mass spectra are shown of the following:

a) background from the ceramic probe assembly (accurate masses are shown),

b) the same background after exposing the tip to D₂O, and

c) the background after rinsing with methanol.
We have previously described a GC/GC/MS which utilized a flame ionization detector (FID) for detection of the GC effluent from the primary chromatograph (Ligon and May, J. Chromatogr. Sci., 24, 2 (1986)). This configuration required that 10% of the primary effluent be split to the FID which reduced ultimate MS sensitivity. Conversely, the splitting produced a 90% loss in FID sensitivity.

We have now modified our apparatus so that the FID is replaced by a photoionization detector (PID). In this configuration all of the primary effluent flows through the detector. The detection of minor components in the primary effluent is much enhanced. In addition, sample losses due to ionization in the PID are very small compared with the 10% loss of the FID. We have encountered only small losses in GC resolution in passing through the PID. In addition, we find that the PID cell is unaffected by the 10-20 PSI of internal pressure which it encounters during the secondary separation.

We have found that the relatively large quantities of material which reach the PID during 2D separation causes it to become contaminated relatively quickly. In addition, we find that using the standard 10.2 eV lamp, the PID is a relatively selective detector (e.g. poor response to saturated hydrocarbons) compared with an FID and this is a handicap in some analyses.

![Diagram of GC apparatus using a photoionization detector](image)

**Figure 1.** Two dimensional GC apparatus using a photoionization detector for recording the primary chromatogram.
Prudhoe bay crude oil
100 μg on column

conditions:
30 m DBwax-megabore
Program: 50-230 @ 12 C/min
Detector: PID 10.2 eV lamp
Chart: 10 mm/min

Figure 2. Primary chromatogram for Prudhoe bay crude oil obtained using the PID.

Figure 3. Secondary chromatogram for "Cut A" from the primary separation shown in Figure 2.
Laser desorption of neutral molecules in combination with multiphoton ionization and time-of-flight mass analysis has become a well-known technique to examine non-volatile, labile molecules [1]. Instead of multiphoton-ionization, it is also possible to use other ionization techniques, e.g. electron-impact (EI) ionization. EI is able to ionize molecules with higher ionization potentials, which can practically not be photon ionized with satisfying sensitivity. But on the other hand EI is said to be the less sensitive method, much more fragmentizing and therefore not so attractive for the analysis of labile species.

The measurements have been performed with a Bruker TOF-1 including EI ionisation unit. Neutral molecules are vaporized by pulsed infrared CO$_2$-laser radiation (10.6 μm, 10 μs). The desorbed intact molecules penetrate a pulsed supersonic jet (4 bar, 500 μs), which cools them and carries them into the ionization region. Here the neutral molecules are bombarded with 20 eV electrons for several μs. The ions produced are trapped in the focus of the electron beam. After the electron beam is switched off, the ions are pulsed into the TOF mass spectrometer. This technique allows resolutions of m/dm = 2000 (FWHH) and sensitivities not much less than that of multiphoton ionization (MPI). For samples with higher ionization potentials and exited states not matched by the photon energy, however, the sensitivity of EI-ionization is much higher than that of multiphoton ionization. The apparatus allows fast switching between both ionization techniques for direct comparison.

We have analyzed several peptides in the mass range up to 2000 u with the general finding that for all those species, where the molecular ion can be observed with multiphoton ionization (MPI), it also can be produced with electron impact ionization. EI often produces fragments similar to those produced by MPI, which leads to the conclusion that fragmentation is already "predecided" during desorption, although it is finally executed during ionization. For larger peptides, however, the fragments are more characteristic with MPI. Fig. 1 shows a complete raw data spectrum of Gramicidin D, the molecular ion is clearly seen. For Gramicidin S (see Fig. 2) the dimer and trimer are observed, too. The peptide PRO-LEU-GLY in Fig. 3 does not contain any double bonds, and therefore can not be ionized with MPI, if, as in our case, only laserlight in the range 230 - 300 nm is available.

The three stage ion source ND-jet-EI (Neutral Desorption - jet cooling - Electron impact Ionization) is well-suited to produce ions of labile, non-volatile compounds up to 2000 u. The success of this ion source is mainly based on the jet cooling of the desorbed ions as discussed in a parallel study [2]. EI ionization, when performed as described above, is a good and cheap alternative to multiphoton ionization. The best results can be obtained when EI and MPI are available, using the advantages of both methods alternatively.

Fig. 1: Electron impact ionization mass spectrum of Gramicidin D (raw data, electron energy 20 eV, jet gas neon)

Fig. 2: Electron impact ionization mass spectrum of Gramicidin S (raw data, electron energy 20 eV, jet gas neon)

Fig. 3: Electron impact ionization mass spectrum of PRP-LEU-GLY (electron energy 20 eV, jet gas neon)
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SHORT CAPILLARY COLUMN GC/FID UNDER VACUUM OUTLET CONDITIONS AND ITS RELEVANCE TO GC/MS

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Introduction
Short open tubular capillary columns can be employed in GC/MS when rapid separation is desired prior to mass spectral analysis. The effects of vacuum outlet operation are much greater for short columns (less than 10 meters) than for long columns. As the column length is decreased, the optimum velocity under vacuum outlet operation increases significantly. Thus, the most obvious advantage of short columns with vacuum outlet is the improved speed of analysis. Corresponding to this advantage is a loss in the number of theoretical plates by a factor of 8/9 versus atmospheric outlet operation.

The chromatographic performance of short columns may be studied by vacuum outlet GC/FID under approximately the same conditions as GC/MS. Previous theoretical calculations have shown that the pressure drop along a short column for a 100 torr outlet pressure closely resembles the pressure drop for subtorr outlet pressures [1]. Thus, the chromatographic performance (evaluated by Golay plots) of 140 torr vacuum outlet GC/FID and of GC/MS should closely resemble each other.

Experimental
Experiments with flame ionization detection were performed on a Varian 3400 GC with modifications to the injection port and detector tower to allow for both sub-ambient inlet and outlet operation. Experiments with MS detection were conducted on a Finnigan TSQ45 with a 9610 GC. The injection port was modified to allow for sub-ambient inlet operation. The comparison between GC/FID and GC/MS consisted of analyzing dodecane isothermally at 60°C, over a range of inlet pressures (linear velocities). A mixture of n-alkanes (C_{14}, C_{15}, and C_{16}) was analyzed by the GC/FID system by adjusting the inlet pressure for each chosen outlet pressure so as to run the chromatograms at the optimum carrier gas velocities.

Results and Discussion
The retention times of dodecane, analyzed by both GC/MS and vacuum outlet GC/FID (140 torr), showed excellent agreement between the two methods, as well as with theoretical expectations. An extra-column band broadening of 0.6 seconds was evident in the GC/MS data, whereas the GC/FID data appeared to have only a minor amount of band broadening. It is thought that the calculated linear velocities in the GC/FID study are lower than the linear velocity actually present; this deviation results due to the location of the pressure gauge, which is removed from the injection port (located between the split and injector buffer). Thus, the observed inlet pressure is less than the true pressure resulting in lower velocities when calculated by the Poiseuille equation:

$$u = \frac{3(r)^2P_0(P_0P - 1)^2}{32\eta L(P_0P - 1)}$$

where $u$ is the average linear velocity in cm/s, $r$ is the column radius in cm, $P_0$ is the outlet pressure in dyne/cm$^2$, $P$ is the ratio of inlet to outlet pressures $P_i/P_0$, $\eta$ is the
carrier gas viscosity in poise and L is column length in cm. By the analysis of plate height versus linear velocity (i.e. a Golay plot), one may find the optimum linear velocity and plate height at any given velocity. Figures 1 and 2 are the Golay plots for GC/FID and GC/MS respectively. On Figure 2, both theoretical Golay curves (with and without the 0.6 second band broadening) have been plotted along with experimental data.

Loss in resolution under vacuum outlet operation has been studied on the GC/FID system. A summary of the results, including running conditions (Table 1) and loss or resolution (Table 2) appears below. It is of interest to note that the chromatograms run at 400 and 140 torr required sub-ambient inlet pressures in order to achieve the optimum linear velocities. Table 2 presents the loss of resolution versus atmospheric outlet operation for the various chromatograms which were run at different outlet pressures. At 140 torr, the experimentally determined loss of resolution of approximately 6% is in excellent agreement with the theoretically predicted value of 6%. The chromatograms demonstrate the corresponding improvement in the speed of analysis (by a factor of 2).

Modeling of Diffusion and Sputtering Processes in a Glow Discharge Cell

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Glow discharge (GD) cells are used both in optical and mass spectrometric ways of elemental trace analysis. In order to understand and describe one of the basic processes in a GD cell, a one-dimensional sputtering model was established [1] utilizing the Boltzmann equations for ions, neutrals and electrons [2]. A critical detail we have learnt from the model was the relative importance of redeposition processes due to back-diffusion of sputtered neutrals directed towards the sample surface.

Here we report the extension of the diffusion model into three dimensions providing the possibility of tracing both axial and radial processes for real cell configurations. The source term of the model couples the diffusion processes to sample erosion through thermalization profiles of sputtered neutrals. The diffusion equation, in cylindrical coordinates, for a steady state glow discharge plasma then becomes:

$$\frac{\partial n(r,z)}{\partial t} = D \left[ \frac{\partial^2 n(r,z)}{\partial r^2} + \frac{1}{r} \frac{\partial n(r,z)}{\partial r} + \frac{\partial^2 n(r,z)}{\partial z^2} \right] + J_0 F_r(z)$$

where $n(r,z)$ is the sputtered neutral atom density (atoms/cm$^3$), $D$ is the diffusion coefficient (cm$^2$/sec), $J_0$ is the sputtered atom flux (atoms/cm$^2$sec) and $F_r(z)$ (cm$^{-1}$) is the normalized thermalization profile of the sputtered neutrals. $J_0$ and $F_r(z)$ are calculated according to the sputtering model described in refs [1] and [2]. Solutions of this differential equation are sought with the aid of a computer code [3] using a finite difference method, together with the appropriate boundary conditions. In this way, the analytical performance of different simple cell designs can be investigated theoretically, assuming a constant ionization efficiency throughout the cell if it is coupled with a mass spectrometer. Overall, the performance of a cell is thought to be influenced by the axial transport whereas cell wall contamination may be correlated with radial spread of material.

Fig. 1 shows the sputtered neutral atom density variation in two different cell designs. The standard cell used in our mass spectrometer for analyzing flat samples has a "neck"-formed anode body to constrict the discharge to a part of the cathode sample. This design can simply be improved, by cutting off a part of the neck and by increasing the radial distance to the cell walls, as is shown in Fig. 1b. In this way, the axial diffusion profile of the sputtered atom number density shows a distinct increase of atom density close to the exit slit, situated at the back of the cell (Fig. 2). Also the diffusion flux towards the end of the cell increases by a factor of 2.5, which can be estimated from the slope of the diffusion profile close to the exit slit. Another possible improvement would be the use of a sampling cone immersed into the cell interior (not shown here). The disturbance of the equidensity lines in this case also creates a larger diffusion flux. Additionally, the distortion of the electrostatic fields outside the cell improve the focussing properties of the electrostatic lens system in a mass spectrometer. This feature will be illustrated elsewhere [4].
Fig. 1: Neutral atom density map for the standard cell (a) and a modified cell (b). The sample surface is situated on the left of both figures (z=0, -0.4 ≤ r ≤ 0.4), the exit slit on the right.

Fig. 2: Axial density profile (along z, for r=0) for the standard cell and the modified cell, showing a larger neutral atom density and diffusion flux close to the exit slit for the modified cell.

Glow discharge mass spectrometry (GDMS) has become a viable method for trace elemental analysis of solid conducting materials (1). In most applications of the technique, argon is used as the discharge gas. However, under certain conditions the argon complexes with various species to form complex ions that can result in isobaric interferences. One method of removing these interferences is to use an alternative discharge gas, such as neon, which will shift the m/e value of the complex ion. With the increasing application of GDMS, the use of neon discharges can be expected to proliferate and fundamental information on excitation processes in neon discharges would be valuable. Three ionization mechanisms have been most commonly reported to be responsible for the ionization of sputtered atoms; electron impact ionization, charge exchange ionization, and Penning ionization. Electron impact ionization results from an energy transfer from an energetic electron, charge exchange ionization can occur when an energy level for an ion of the rare gas directly overlaps with an energy level of the sputtered atom, allowing the transfer of an electron, and Penning ionization results from a potential energy transfer from an excited metastable state of the discharge gas.

Several previous studies of low pressure, low current argon glow discharges have shown the argon metastable atoms to significantly impact the extent of ionization in the discharge through the Penning ionization process (2,3). Neon discharges would be expected to behave in the same manner, but little direct evidence of Penning ionization in neon glow discharges has been presented. These experiments were designed to quench the metastable levels of the neon and measure the resulting decrease in ion emission from a sputtered copper population, employing a diode geometry glow discharge operating at 0.5-5 torr and 1-5 mA with an optical system discussed elsewhere (2,3). The results for the neon system were then compared to argon in an effort to determine if significant differences in excitation/ionization were present in the two discharge gases.

Methane was employed as the metastable quenching agent. As illustrated in Figure 1, low levels of methane added to a neon glow discharge results in the removal of the Ne* states without significantly impacting the ground state copper population. Under these conditions, the Penning ionization process should be removed, allowing ionization only through electron impact, assuming there is no energy overlap for charge exchange to occur. As shown in Figure 1, a reduction in ion emission signal also occurs with the introduction of methane, indicating the Ne* does play a role in the ionization processes in the glow discharge. Discharge pressure is also known to influence the population of various discharge species, and the pressure profiles of different species provide one method of illustrating the role of metastables in discharge ionization. Figure 2 shows the population of the ground state copper atoms and neon metastables as the discharge pressure is varied. The neon metastables show a peak at ~1.8 torr, while the Cu° population decreases linearly with increasing pressure for both a neon and 10% methane/neon mixture.
Figure 3A exhibits the effect of pressure on three copper ion emission signals for both pure neon and a 10% methane/neon mixture. The role of Penning ionization is clear in this figure, with the ion emission signal profiles in neon peaking at 1.8 torr, matching the behavior of the Ne* population (Figure 2). With methane present in the discharge, 3B, the ion signal is reduced, and the profile is closer to that of the copper ground state (Figure 2). These same transitions were monitored in argon and 10% methane/argon under as similar conditions as possible to compare directly the extent of ionization with each discharge gas. The results are presented in Figure 4A for pure argon and 4B for the methane mix, plotted relative to the maximum signal for neon. The neon provides significantly higher ion emission signals for these transitions and the enhancement is removed upon metastable quenching. This is most likely due to the role of Ne* in the population of the upper energy level of these transitions. The neon metastable has sufficient energy (16.62 and 16.67 eV) to populate these levels directly (15.91 -16.52 eV), while argon metastables (11.55 and 11.72 eV) will require supplemental energy and a less likely two step process to populate these levels. Penning ionization to these levels will be enhanced in neon, but as the metastables are quenched, so will the Penning ionization process, and the enhancement with neon will be removed. Zinc ion absorption data employing a transition originating from a ground state ion level (20 nm) showed no enhancement with neon, and the methane quenching effects were approximately equal for both discharge gases. These results indicate the enhancement holds for specific excited state ion levels, not necessarily the bulk ion population, a fact consistent with the postulated role of the neon metastable in the excitation/ionization processes in glow discharges. Further investigations include studying a possible energy level dependence on the neon enhancement, comparing other discharge gases and cathodes, and studies of laser excitation and depopulation in the discharge.

References:

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As an elemental analysis technique, glow discharge mass spectrometry (GDMS) combines the simplicity of design of the glow discharge ion source with the high sensitivity and selectivity of mass spectrometry. As with any technique, certain limitations have arisen to challenge existing methodology; an example of this can be found when considering the problem of isobaric interferences. Shown to be a universal problem in elemental mass spectrometry, isobaric interferences arise from several sources including overlapping isotopes, doubly charged species, and polyatomic ions; some typical glow discharge diatomic ion interferences include dimers, oxides and argides. Two schemes that have been used to deal successfully with these interferences are discrimination and suppression/elimination. The first of these is the approach of high resolution mass spectrometry. Although commercial instrumentation is capable of resolving \( m/\Delta m_w = 10000 \), the required resolution necessary to effect separation of these species from an analyte of the same nominal mass can range to an excess of 60000. 

Recently we have interfaced a glow discharge-ionization source to a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer. Utilizing the ultra high resolution capabilities of the FTICR approach (resolution in excess of 100000), we are capable of more successfully addressing the problems that typical diatomic and polyatomic interferences pose in GDMS.

**EXPERIMENTAL**

Positive ions generated in the negative glow region of a coaxial cathode glow discharge were mass analyzed by a home-built FTICR mass spectrometer that employs Nicolet FTMS-1000 electronics in controlling a 4.50 cm x 2.54 cm x 2.54 cm ion analyzer cell. A series of four electrostatic lenses acts to focus and accelerate ions sampled from a 1.0 mm diameter ion exit orifice, and a cylindrical decelerator serves to slow the ion beam prior to injection into the analyzer cell. The trapping plates were dropped from +2V to ground for a period of 0.1 - 5.0 seconds to allow ions to be "injected" into the cell, and then raised back to +2V for the detection phase. In typical experiments ions with frequencies from 10 kHz to 2 667 MHz were excited and detected with a bandwidth of 2.667 MHz. Fifty time domain signals of 16 K data points each were accumulated, and the average time domain signal was apodized by a modified Blackman-Harris window function and zero-filled once prior to Fourier transformation. For higher resolution analysis, fifty time domain signals of 64 K data points each were averaged, and a standard heterodyne approach was employed.

**RESULTS AND DISCUSSION**

Although the FTMS approach holds the potential for both discrimination (with ultra high resolving power) and elimination (with a greater probability for collisional dissociation than the TSQ approach) of isobaric interferences, we are presently concentrating our efforts on the discrimination aspect. In a series of precursory studies, moderate resolution mass spectra were generated from a compacted silver cathode in an argon discharge. Figure 1 illustrates the 106 - 110 a.m.u. region of the mass spectrum with Ag\(^+\) indicated at nominal masses of 107 and 109. The observed resolution for 107 Ag\(^+\) is 5996 (full width at half maximum). Although this spectrum shows only moderate resolution, our highest resolution spectrum obtained to date, (Figure 2: \( m/\Delta m_w = 42549 \), for the Ar\(^+\) ion) demonstrates the possibility for using ultra high resolution to discriminate against glow discharge isobaric interferences.

One limitation to the GD-FTICR approach, thus far, is its poor sensitivity compared to other GDMS instruments. Since most isobaric interferences only begin to appear at the 100 ppm level, we are somewhat limited by our present detection limit of 25 ppm. However, we believe that the
future addition of a Bessel Box energy analyzer and quadrupole ion guide should help improve ion transport capabilities and lower the L.O.D by several orders of magnitude.

![Graph](image)

**Figure 1.** Moderate resolution GD-FTICR mass spectrum of a silver cathode in the mass range 106 - 110 amu; \( m/\Delta m_w = 5996 \) at nominal mass \(^{107}\text{Ag}^+\).

**Figure 2.** High resolution GD-FTICR mass spectrum in the mass range 39.96 - 39.97 amu; \( m/\Delta m_w = 42549 \) at nominal mass \(^{40}\text{Ar}^+\).
The analytical technique of trace elements is required to detect at least ppt level of impurities. However, various artifact ion species generated in the ICP interface are isobaric with the atomic ions from the elements to be determined, and thus the determination of trace quantities of these elements is not generally possible. Artifact ion species are formed by the reaction of carrier gas (Ar) and the oxygen originated from aqueous samples. We discuss the ICP-high resolution mass spectrometer which is able to identify the ion component at minimal intensity level.

Experimental

It is known that most of the artifact ions can be resolved from the elemental ions by operating the mass spectrometer at a resolving power of 10,000. For this purpose, we have developed an ICP-high resolution mass spectrometer (ICP-HRMS) based on the QQBQ.E geometry which has been used for organic mass spectrometer, which is able to obtain resolution up to 60,000 by EI. Data system program for ICP-HRMS has both low and high resolution measurement capabilities. The used torch assemblies, coaxial nebulizer and spray chamber are the same as conventional type. Radio frequency power 40MHz generator is ICP16L (Plasma Products Inc., USA). A new interface has developed to obtain high ion transmission efficiency and non-arching at high accelerating voltage (Fig.1). The evacuation system has consist of two stages that have RP and TMP after sampling cone and skimmer cone, respectively. The glow discharge in the RP stage could avoid by increasing the length of insulated pumping hose to 1.8m at accelerating voltage 5kV.

Results

ICP-HRMS spectrum was measured by magnetic field scanning from m/z 1 to 85 including elements of Li, Be, Al, Cr, Fe and Ni; each concentration 100ng/ml in nitric acid (1mol/l), under the condition of resolution 4,000 (Fig.2). In the expanded mass spectra are able to show the good separation of element ion peaks and artifact ion species peaks. For example, the partial range mass spectra are showed in figure 3(a) - (c), and elemental compositions of all peaks are assigned. And figure 4 shows the expanded mass spectrum at m/z 56, 10ppb Fe and ArO, under higher resolution condition R=10,000.

Conclusions

Masses and isotope abundances of element and artifact ion species produced in ICP interface could be determined by using HRMS, we found that they are multi-atomic ion complex consist of Ar, O, N and H. Especially, it is necessary to identify 2nd and 3rd abundant isotope such as \(^{16}\)N, \(^{17}\)O, \(^{18}\)O, contribution for the analysis of trace level of elements. The results of this study suggest that the high resolution, high sensitivity ICP-HRMS is very effective means to obtain reliable qualitative and quantitative results.

References

Figure 1. A SCHEMATIC DIAGRAM OF THE ICP INTERFACE FOR THE HIGH RESOLUTION MASS SPECTROMETER.

Figure 2. THE ICP-HRMS MASS SPECTRUM OF Li, Be, Al, Cr, Fe, AND Ni, 100ng/ml EACH IN NITRIC ACID (1mol/l).

Figure 3. THE EXPANDED PARTIAL RANGE MASS SPECTRA OF (a) Li, (b) Cr AND (c) Ni.

Figure 4. THE ICP-HRMS MASS SPECTRUM OF IRON AND ARGON OXIDE 5 PPM IN 5% HNO₃.
High Resolution ICP-MS Investigation of molecular species In difficult matrices.
A Walsh, N Bradshaw, R C Hutton, J E Cantle. VG Elemental, Winsford, Cheshire,

Introduction

The use of the argon inductively coupled plasma as an ion source has become widely accepted in inorganic and elemental mass spectrometry. Approximately five hundred systems are now in use worldwide. Conventional ICP-MS instruments couple the atmospheric ICP to a quadrupole mass spectrometer via an optimised supersonic beam interface.

The ICP is well suited to use as an elemental ion source. The relatively high first ionization potential of argon (15.7eV) gives greater than 90% ionization for over 70% of elements within the plasma, however the levels of doubly charged ions are minimized. The ions are sampled from a region at approximately 8000K, thus ensuring high levels of dissociation and ionization even for refractory materials.

The spectrum produced from the ICP is simple, consisting of mainly isotopic peaks. However matrix specific interferences can limit the determination of some elements at trace and ultratrace levels due to spectral overlaps, when measured with the quadrupole instrument. These matrix related peaks occur mainly at lower mass and are predominantly singly charged two and three atom species. The formation processes for these species are not well understood and the normal method of overcoming particular problems has been to reduce the level of the problematic matrix element. For example it is received wisdom that the use of sulphuric and hydrochloric acid based digestion should be avoided and replaced by nitric acid, to reduce the occurrence of such interfering species.

We have investigated the formation of molecular species in two common matrices, sulphuric and hydrochloric acids. The behaviour of molecular species with matrix concentration has been determined. Data has been obtained showing how the use of a high resolution mass analyser in place of the quadrupole allows the analysis of interfered elements in the so called difficult matrices. The high resolution mass analyser also allows the identification and separation of molecular species appearing at nominally the same mass in the spectrum.

Instrumentation

The investigation was undertaken using the VG PlasmaTrace high resolution ICP-MS. This instrument couples the ICP to the double focusing magnetic sector mass spectrometer via a novel plasma sampling interface (pat pending) that extracts ions from the ICP and accelerates them to the high energies required by a double focusing mass spectrometer of this type. Instrument resolution can be quickly varied by means of remotely controlled variable slits at the object and image points of the spectrometer.

The very low background noise of the instrument (typically 0.1 Hz) allowed confirmation of species formation even at very low equivalent concentrations. In the acid interference investigation the instrument resolution was set to 3400 to allow for separation of the potential molecular species formed at nominally the same mass to be monitored. Resolution used during the quantitation of known levels of trace elements in both hydrochloric acid and brine solutions was varied during the analysis to resolve expected interference species.

Sample introduction was by conventional pneumatic nebulization using a corrosion resistant cross flow nebulizer. A peristaltic pump was used to introduce solution to the nebulizer to overcome any problems with sample viscosity changes. A platinum sampling cone was also used in this work as the conventional nickel cone was rapidly eroded by high acid concentrations.
Experimental

1. Acid Matrices

The masses of a number of known and potential interferences were monitored for a series of solutions of different concentrations of sulphuric and hydrochloric acid. Indium at 115 amu was used as an internal standard to monitor instrument stability and to correct for any potential suppression of instrument response with higher acid concentrations. Peak integrals were determined for all ions detected.

2. Quantitative Analysis of chloride matrices

Standard solutions of 0.05N HCl and 10 g/l of NaCl of known elemental concentration were analyzed. Resolution was selected so to allow the separation of predicted spectral interferences from the isotopes of interest. Three external standards of 10, 50 and 100ng/ml were used.

Conclusion

The VG PlasmaTrace has been designed as a variable resolution instrument to allow the analyst to achieve the best possible detection limits by optimising resolution to resolve isotopic and molecular peaks only when present in the spectrum and to use the low resolution highest sensitivity mode for isotopic peaks with no interference.

We are investigating the development of an automated pre-analysis routine to determine the sample matrix and the use of a simple expert system to predict the isotopes likely to be interfered by molecular species derived from the matrix constituent elements. The instrument would then be automatically configured to measure the user selected elements of interest at the resolution required for the particular matrix. To maintain total flexibility the user would have the capability of overriding this procedure.

Such a system requires that a rule set be produced to predict the likely interferences for any matrix. The underlying purpose of this work was to begin to attempt to identify that rule set.

The data shows that as might be expected the intensity of the molecular peaks does increase with matrix concentration. The trend appears to be linear. A number of the monitored masses showed no evidence of the formation of proposed species even at relatively high acid concentrations, whilst all the species that were detected occurred even at the 1% acid level. This allows the conclusion that, within the detection capability of the instrument and in these matrices, molecular formation is not triggered by the density of constituent elements being introduced into the plasma. Further studies are required to confirm this at matrix concentrations below those studied.

Two, three and four atom species have been positively identified by accurate mass assignment at medium and high resolution. Given the high thermal temperature (8000K) of the ICP together with the sample residence time within the plasma, it is unlikely that any molecular species would survive introduction into the ICP, to be sampled by the supersonic beam system. The supersonic expansion that then occurs means that the adiabatic motion of particles within the ion source that is formed is rapidly and efficiently transformed into isentropic motion along the beam axis. Therefore the collision frequency rapidly tends to zero. It is unlikely that three (and four) body collisions could occur in this region. It is proposed that reactions on the surfaces of the interface must be of major importance in the formation of molecular ions.

We have been unable yet to correlate the species formed with any predictive model, further work is required with a much larger set of matrices to attempt to identify this important model to allow both a simple predictive procedure to be used and also to allow the further development of the sampling interface to minimize the formation of molecular species.
Fast Atom Bombardment Mass Spectrometry of Iron-Sulfur Cubane Clusters

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The cubane cluster \([\text{Fe}_4\text{S}_4]\), is one of the most widely distributed electron transfer sites in biology. Synthetic analogs have been investigated by an intensive body of structural, spectroscopic and magnetic studies for the last two decades\(^1\). However, no systematic study exists to determine either the suitability of FAB-MS, or the proper conditions necessary, for the characterization of these complexes. In the present work, both positive and negative ion FAB-MS has been used to characterize a series of iron-sulfur cluster dianions \([\text{Fe}_4\text{S}_4\text{X}_4]^2-\) with different cations: (a) \((\text{A})_2\text{Fe}_4\text{S}_4\text{Br}_4\), \(\text{A}=\text{Bu}_4\text{N}, \text{Pr}_4\text{N}, \text{Et}_4\text{N}; \) (b) \((\text{A})_2\text{Fe}_4\text{S}_4\text{Cl}_4\), \(\text{A}=\text{PPN}, \text{Ph}_4\text{P}, \text{Bu}_4\text{N}, \text{Me}_4\text{N}; \) (c) \((\text{A})_2\text{Fe}_4\text{S}_4(\text{SEt})_4\), \(\text{A}=\text{Ph}_4\text{R} \) \(\text{Ph}_4\text{As}; \) (d) \((\text{A})_2\text{Fe}_4\text{S}_4(\text{SPh})_4\), \(\text{A}=\text{Ph}_4\text{P}, \text{Bu}_4\text{N}; \) and (e) \((\text{Ph}_4\text{P})_2\text{Fe}_4\text{S}_4(\text{SPh})_2\text{Cl}_2\), where \(\text{Ph}=\text{Phenyl}, \) and \(\text{PPN}=(\text{Ph}_3\text{P})_2\text{N}.\)

Both 3-nitrobenzyl alcohol (NBA) and 2-nitrophenyl octyl ether (NPOE) are suitable matrices for the FAB-MS studies of complexes (a) and (b). Combining the structurally informative positive- and negative-ion spectra of \((\text{Et}_4\text{N})_2\text{Fe}_4\text{S}_4\text{Br}_4\) (Fig. 1 and Fig. 2), a molecular weight determination of iron-sulfur cluster complexes has been successfully achieved. Most of the complexes of (a) and (b) give similar mass spectral features to those shown for \((\text{Et}_4\text{N})_2\text{Fe}_4\text{S}_4\text{Br}_4\).

The positive ion FAB-MS studies of (c) and (d) give very limited information related to the molecular formula, however negative-ion measurements of all clusters using the matrix NPOE, provide the unique features of molecular ion and rich cluster fragment ions analogous to those found in the systems (a) and (b). Interestingly, if NBA is used as a matrix, a terminal ligand substitution reaction between NBA and \((\text{A})_2\text{Fe}_4\text{S}_4(\text{SEt})_4\) occurs, as shown in Fig. 3.

A mixed ligand cluster \((\text{Ph}_4\text{P})_2\text{Fe}_4\text{S}_4(\text{SPh})_2\text{Cl}_2\) was chosen to test the usefulness of FAB-MS to identify the disproportionation species of a ligand exchange equilibrium system shown in eq 1-3. FAB analysis (shown in Fig. 4) confirm these different species in solution except for \([\text{Fe}_4\text{S}_4(\text{SPh})_4]^2-\), which suggests the presence of the equilibria of eq 1 and eq 2 are predominant rather than those of eq 3.

\[
\begin{align*}
2[\text{Fe}_4\text{S}_4(\text{SPh})_2\text{Cl}]^2- & \underset{\text{eq } 1}{\longrightarrow} [\text{Fe}_4\text{S}_4(\text{SPh})\text{Cl}_3]^2- + [\text{Fe}_4\text{S}_4(\text{SPh})_3\text{Cl}]^2- \\
[\text{Fe}_4\text{S}_4(\text{SPh})_3\text{Cl}]^2- & \underset{\text{eq } 2}{\longrightarrow} [\text{Fe}_4\text{S}_4(\text{SPh})_2\text{Cl}_2]^2- + [\text{Fe}_4\text{S}_4\text{Cl}_4]^2- \\
2[\text{Fe}_4\text{S}_4(\text{SPh})_2\text{Cl}]^2- & \underset{\text{eq } 3}{\longrightarrow} [\text{Fe}_4\text{S}_4\text{Cl}_4]^2- + [\text{Fe}_4\text{S}_4(\text{SPh})_4]^2-
\end{align*}
\]

The results demonstrate that FAB-MS can be employed as a valid and convenient method for molecular weight determination and structural elucidation of iron-sulfur cubane clusters, as well as identification of intermediates of ligand substitution and disproportionation of mixed ligand cluster in the solution. The choice of matrix however is critical.

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Figure 1. Positive-ion FAB mass spectrum of \([\{(Et_4N)_{2}Fe_4S_4Br_4\}]\) In NBA.

Figure 3. Negative-ion FAB-mass spectrum of \([\{(Ph_4P)_{2}Fe_4S_4(SEt)_4\}]\) In NBA.

Figure 2. Negative-ion FAB mass spectrum of \([\{(Et_4N)_{2}Fe_4S_4Br_4\}]\) in NPOE.

Figure 4. Negative-ion FAB-mass spectrum of \([\{(Ph_4P)_{2}Fe_4S_4(SPh)_2Cl_2\}]\) in NPOE.
A considerable interest has been focused on the preparation and screening of transition metal complexes for use as possible chemotherapeutic agents since the first study reported in late sixties that the flat platinum (II) complex cisplatin shown successful evidence to against cancers. The mechanistic studies have also shown that the transition metals in these complexes provide a key rule in binding of the complexes to DNA. Recently, studies also shown the interactions between some square planar and planar orthometallated palladium (II) complexes and DNA. The most interesting activity towards DNA of the orthometallated complexes was exhibited by (N,N,2C')-2(6'-methoxyphenyl)-palladium (II)-chloride. The binding of this complex to DNA was reversed by the addition of NaCN, which suggested that ionization of Pd-Cl bond in the aqueous reaction medium was necessary for its binding to DNA.

It is suggested [1] that o-thiocresol derivatives of orthometallated Pd(II) complexes can act as a monitor in the event of a radical-based Pd-C homolysis. The abstractable benzylic C-H bond will quench the radical, forming a thio-metallacycle. To study the formation of thio-metallacycle, a series of thiocresol-Pd(II) complexes were prepared. The structures of these derivatives are listed as follows. Mass spectral analysis of these complexes was performed using fast atom bombardment (FAB) mass spectrometry which has proven to be a versatile ionization method for the analysis of inorganic, organometallic and coordination compounds [2].

FAB mass spectra were obtained from a Kratos MS-80 double focusing mass spectrometer operating at an accelerating voltage of 4 kV. A fast atom saddle-field gun (Iontech FAB-11NF) is mounted on a standard Kratos FAB source. The xenon atom beam was generated with a kinetic energy of ca. 6 kV.

It is noticed that these complexes do not generate FAB mass spectra under normal operation conditions using conventional liquid matrices such as glycerol, thioglycerol and 3-nitrobenzyl alcohol (NBA). Instead of introducing a new liquid matrix which may increase the solubility of metal complexes, it was found that a key point for obtaining FAB mass spectra of these complexes is the pretreatment of samples. The pretreatment of samples was very simple: firstly, add approximately 20 μg of sample into 10 μl of methanol, stir it to form a colloid and remove the colloid onto a FAB probe tip; then place 1 μl of NBA matrix on the tip before methanol was totally evaporated, stir it again and wait approximately 5 minutes to perform mass spectral analysis.

FAB mass spectra of some thiocresol-Pd(II) complexes are shown in Fig. 1. The molecular ion cluster was observed as a major peak at high mass region. The other major peak at high mass region corresponds to a fragment formed by the cleavage of Pd-S bond. Compare to the orthometallated Pd (II)-Cl complexes which generate a fragment resulted from Pd-Cl bond cleavage as the highest mass, the Pd-S bond seems to be stronger than the Pd-Cl bond. It is because the Pd-Cl bond was enhanced by the formation of a thio-metallacycle.

Interestingly, the predominant ions observed at molecular ion region in FAB mass spectra of these complexes correspond to M⁺, rather than the protonated [M+H]+. However, the protonated molecular ion was still observed, but its intensity is lower than that of molecular ion. It is clearly shown in Fig. 2 that a peak at m/z 524 corresponds to the molecular ion with most abundant Pd isotope (a.m. 106, natural abundance 27.3%). Since Pd doesn't have an isotope with a.m. 107, the intensity of peak at m/z 525 should only be about 24% of that of m/z 524 (contributed from 13C isotope). A relative intense peak observed at m/z 525 would indicate that there still undergoes a protonation process, even though it is only a minor
process. A plausible explanation for this observation is that these complexes do not have a stable protonation site. The formation of thio-metallicycle prevented the heterocatom sulphur from being a protonation site. On the other hand, the oxygen atom, if any, in the distal R group were not a suitable protonation site either. This would agree with our recent study [3] that a certain group of compounds do not generate protonated \( [M-H]^+ \) as predominant ions under FAB conditions.

REFERENCES


Fig. 1a.

Fig. 1b.

Fig. 2.
Investigation of Multiply Charged Binuclear Metal Complexes Capable of Small Molecule Activation: Alex Reiter, Kristin Bowman Mertes, Tamboue E. Deffo, and Todd D. Williams; University of Kansas, Lawrence, KS 66045.

Introduction

We have synthesized asymmetric macrocycles capable of coordinating two different metals (Co, Cu, Mn, Ni, and Zn) and symmetric macrocycles for small molecule activation, e.g., CO2 and O2. The best method of characterizing these compounds has been mass spectrometry. FAB and B/E linked experiments were used to confirm the structure and to try to determine the location of the metals in the asymmetric macrocycle. FAB was used to characterize the interaction between the macrocycle and CO2 or O2.

Macrocycles used in the study

Experimental

Mass spectra were obtained on a VG ZAB-HS mass spectrometer equipped with an 11/250 data system. Fast-atom bombardment experiments were performed using a Xenon gun operated at 8 keV energy and 0.8 mA emission. Non-mixed and single metal samples were dissolved in CH2Cl2 and added to NBA as the matrix. The mixed-metal sample were dissolved in either CH2Cl2 or DMF and stearic acid was added in 1:1 molar ratio. The mixture was then added to NBA as the matrix. A narrow sample range, 12 μg-17 μg on the probe, was required for molecular ion detection. B/E scans were performed with precursor ions attenuated to 30% with He in the first field free region gas cell. Collision spectra were recorded in multichannel analyzer mode (MCA) under data system control. The scan range was 700-5 amu acquired at 15 sec/dec and 3 scans were obtained. The collision energy was 8 keV. The deconvolution were done by minimizing the absolute deviation of the isotope cluster.

Results and Discussion

Using NBA as a FAB matrix, the binuclear complexes with a single metal type produced a molecular ion corresponding to a singly charged species, even though the complex is formally a 2+ or 3+ compound. The molecular ion cluster is composed of a series of ions, which can be explained by various combinations of simultaneous loss of protons and metal reduction. The mixed metal complexes in NBA showed no ions related to the macrocycle (Figure 1), while in glycerol or magic bullet only the free ligand is observed. Various additives were tried in an attempt to get a molecular ion. Stearic acid and other long chain fatty acids (Figure 2), were successful in obtaining a molecular ion peak.

Stearic Acid Effect on Mixed-Metal Complexes

![Stearic Acid Effect on Mixed-Metal Complexes](image-url)
B/E linked experiments were used to try to determine the location of the metal in the asymmetric macrocycles. Initial results of CAD on the non-mixed metal and the single metal asymmetric complexes indicated the technique would not give conclusive evidence for the location of the metal centers. The problems with the method were low daughter ion resolution and possible ambiguities in assigning fragments (Figure 4). So, deconvolution of the molecular ions obtained by FAB from the mononuclear complexes was done in an effort to determine relative proton counts. Information obtained from these experiments was used to determine which half of the molecule the metal prefers.

The manganese macrocycles have shown ability to bind and activate both oxygen and carbon dioxide. Interaction between CO$_2$ and Cl-sym-Mn$_2$ was confirmed by leaking CO$_2$ into the source (Figures 5 and 6). The results of the experiment suggests that the CO$_2$ was replacing the bridging acetate in the Cl-sym-Mn$_2$ complex. The binding of CO$_2$ was also confirmed by UV-VIS and electrochemical methods. The Cl-sym-Mn$_2$ complex spectra has an ion corresponding to a bridging oxygen. The (Mn-O-Mn) structure was verified by its IR band as described by Wieghardt$^1$. We are now interested in determining whether the other oxygenated complexes observed in other FAB spectra of the different metals are mass spectral induced as suggested by Miller$^2$ or oxygenated complexes.

The National Institute of Health for its support of this work (GM 37577). Mr. Bob Drake of the University of Kansas Mass Spectroscopy Lab for his help in obtaining some of the mass spectra.

References

DETERMINATION OF LEAD IN URINE AND WHOLE BLOOD BY STABLE ISOTOPE DILUTION-GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Introduction

The historical widespread use of lead (Pb) in paint, gasoline and automobile batteries has distributed Pb throughout the environment. Subsequently, sub-clinical Pb poisoning has become a significant health problem. Studies have shown that Pb may have adverse long term effects on cognitive and motor function at blood concentrations that are significantly below the level that gives rise to clinical symptoms. We have developed a stable isotope dilution GC-MS method for determining Pb in urine and blood using lithium bis(trifluoroethyl)dithiocarbamate, Li(FDEDTC), as a chelating agent. Precision and accuracy of isotope ratio measurements at the 10 ng level was determined; memory effect was evaluated and the isotope dilution GC-MS method was validated by determining Pb in urine and whole blood standards.

Experimental

Lead nitrate (70.94 atom% $^{209}$Pb, Oak Ridge National Laboratory) used as an internal standard for isotope dilution was dissolved in deionized water with the addition of a few drops of concentrated HNO$_3$. Dilute solutions of the internal standard were prepared from the stock solution. The internal standard solution was calibrated by reverse isotope dilution GC-MS using a natural Pb primary standard.

One mL of the reconstituted urine sample and 200 nL of the whole blood samples were taken and mixed separately with a known weight of standardized Pb solution, in an amount which yielded an optimum isotope ratio in the mixture. The blood samples after mixing with Pb were treated with 400 μL of 25% HNO$_3$, vortexed, allowed to stand for 10 minutes and the supernates were taken for digestion using HNO$_3$ + H$_2$O$_2$. Urine samples were also digested using HNO$_3$ + H$_2$O$_2$ mixture. The Pb(FDEDTC)$_2$ chelate was formed at pH 3 in an acetate buffer using a 20 mM solution of Li(FDEDTC) and extracted into methylene chloride for GC-MS analysis.

A Finnigan MAT-8230 mass spectrometer with a 10 m DB-1 fused silica capillary column was used for isotope ratio measurements. The Pb isotope ratios were measured in duplicate by injecting 1 μL of the chelate solution and monitoring the isotopic cluster corresponding to the fragment ion (M-L)$^+$ formed by the loss of one ligand. Selected ion monitoring with voltage peak switching was used for data acquisition and isotope ratios were determined by integrating the chromatographic peak areas.

Results and Discussion

The $^{70}$ eV EI mass spectrum of Pb(FDEDTC)$_2$ showed that the fragment ion Pb(FDEDTC)$^+$ was of highest intensity. This most abundant isotopic group at nominal m/z 464 was used throughout these experiments to achieve the highest possible sensitivity. This ion group consisted of nominal m/z values 460, 462, 463 and 464, corresponding respectively to $^{209}$Pb, $^{208}$Pb, $^{207}$Pb and $^{206}$Pb.

The precision of the determination of isotope ratios was evaluated by performing replicate measurements of chelated natural Pb using 10 ng Pb for injection on three different days. The values for the overall precision of major isotope ratios m/z 462/464 and 463/464 were about 3%. There was a memory effect that would affect results when measuring samples with isotope ratios that differ by a
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

a memory effect that would affect results when measuring samples with isotope ratios that differ by a factor greater than 10 (Figure 1). This problem can however be minimized by optimum addition of the internal standard solution. No significant memory effect was observed during the sequential analyses of four synthetic mixtures containing 10 to 40 atom% of \(^{206}\)Pb (Figure 2). The isotope dilution GC-MS method was validated by the quantitation of Pb in the NIST freeze dried urine reference material SRM-2670 and bi-level whole blood toxicology controls from Ciba Corning. Pb concentrations were calculated using all the isotope ratios m/z 460/462, 460/463 and 460/464. The results are shown in the Table 1. There is good agreement among the determined and expected values. The signal-to-noise ratio observed in the reconstructed ion chromatogram indicates a potential limit-of-detection of 0.1 \(\mu g/L\) provided extreme care is taken to minimize contamination from reagents, laboratory and analyst.

Conclusion

Stable isotope dilution GC-MS using Li(FDEDTC) as a chelating agent is a sensitive method for Pb determination in urine and whole blood. The memory effect observed can be minimized by optimum addition of the internal standard solution.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Expected ((\mu g/L))</th>
<th>Determined ((\mu g/L))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>109 ± 4</td>
<td>105 ± 3</td>
</tr>
<tr>
<td>Blood Level 1</td>
<td>207 ± 110</td>
<td>288 ± 5</td>
</tr>
<tr>
<td>Level 2</td>
<td>750 ± 250</td>
<td>805 ± 43</td>
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Table

Figure 1

Figure 2
ISOTOPE DILUTION GAS CHROMATOGRAPHY-MASS SPECTROMETRY
DETERMINATION OF COPPER IN BIOLOGICAL SAMPLES

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Introduction

Growing interest in the use of stable enriched isotopes for studying the bioavailability of trace metals and the easy accessibility of organic mass spectrometers to clinical and biomedical laboratories encouraged us to develop gas chromatography-mass spectrometry (GC-MS) for the determination of trace metals in biological samples. Historically, the main problem preventing the widespread applicability of GC-MS methods to trace metals analysis has been the lack of suitable chelating agents. GC-MS requires volatile chelates which do not show memory or carry-over in the sequential analyses of samples with different isotope ratios at nanogram levels. We have successfully used N,N'-ethylenebis(trifluoroacetyl)acetoneimine, H$_2$(enTFA), as a chelating agent for copper (Cu). It is an essential trace element for several enzyme systems and the two well characterized Cu related diseases are Menke’s Syndrome (due to Cu deficiency) and Wilson’s disease (due to Cu excess).

Experimental

The $^{65}$Cu-enriched copper oxide (99.61 atom % $^{65}$Cu, Oak Ridge National Laboratory) used as an internal standard for isotope dilution was dissolved in 0.5 M HNO$_3$ with heating. Dilute solutions of the internal standard were prepared from the stock solution. The internal standard solution was calibrated by reverse isotope dilution GC-MS using the natural Cu primary standard and preparing the Cu chelate.

One mL of the reconstituted urine or serum sample was mixed with a known weight of standardized $^{65}$Cu solution. Urine samples were digested using concentrated HNO$_3$ and 50% H$_2$O$_2$ while serum samples were first deproteinized with concentrated HNO$_3$ followed by the same digestion. The digested biological extract was dissolved in 0.5 mL of deionized water and 2 mL of ethanol was added. The pH of the solution was adjusted to be greater than 11.5 and the chelating agent was added. The Cu(enTFA)$_2$ chelate was prepared by using a 50 mM solution of H$_2$(enTFA) in CH$_2$Cl$_2$. The reaction mixture was then extracted with 1 mL of CH$_2$Cl$_2$ followed by the addition of 2 mL of deionized water to induce phase separation.

Cu isotope ratios were measured in duplicate, by injecting 1 $\mu$L of the chelate solution and monitoring the group of ions corresponding to the molecular ion. The sample was injected on column, a 10 m DB-1 capillary column. Data were obtained in the selected ion monitoring mode using a Finnigan MAT-8230 GC-MS system operated in the electron ionization mode.

A rigorous evaluation of memory effect was performed by sequentially analyzing solutions of natural Cu and enriched $^{65}$Cu for isotope ratios m/z 395/393 corresponding to $^{65}$Cu/$^{64}$Cu, which differed by a factor of 60. The isotope dilution GC-MS method was validated by determining Cu in NIST urine and serum reference materials.

Results and Discussion

No appreciable carry-over or memory effect was observed for the analyses of two Cu samples with isotope ratios differing by a factor of 60 (Figure. 1). GC-MS method for Cu isotope ratio measurements was also validated by comparing the GC-MS results on four synthetic mixtures with those independently determined by inductively coupled plasma mass spectrometry (ICP-MS). The isotope ratios m/z 395/393 obtained by GC-MS and calculated from ICP-MS data for the four synthetic mixtures agreed within 1%.
Table 1 shows the results on the measurement of Cu in NIST freeze dried urine reference material SRM 2670 and human serum 909. Good agreement of the values determined by isotope dilution GC-MS with the certified NIST values indicated the validity of the present method for the determination of Cu in biological materials.

Conclusion

The results of this work demonstrate that isotope dilution GC-MS using H₂(enTFA₂) as a chelating agent can be used for determining Cu in urine. Results obtained are shown to be accurate and precise. No significant memory effect is seen in the determination of altered isotope ratios. This absence of memory effect not only allows accurate quantitation, but also shows that the technique can be used for isotope ratio measurements in metabolic and bioavailability studies.

<table>
<thead>
<tr>
<th>Sample</th>
<th>NIST (μg/L)</th>
<th>Determined (μg/L)</th>
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<tbody>
<tr>
<td>Urine SRM-2670</td>
<td>370 ± 30</td>
<td>410 ± 30</td>
</tr>
<tr>
<td>Serum SRM-909</td>
<td>1100 ± 100</td>
<td>1000 ± 30</td>
</tr>
</tbody>
</table>

Table 1
ISOTOPE DILUTION GAS CHROMATOGRAPHY-MASS SPECTROMETRY FOR CADMIUM DETERMINATION IN URINE

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Introduction

Cadmium (Cd) widely used in industrial processes is a cumulative toxin with a biological half-life in the whole body exceeding 10 years. Cd accumulates mainly in the kidney. In this paper, we report the development of a stable isotope dilution gas chromatography-mass spectrometry (GC-MS) method using enriched $^{106}$Cd as an internal standard and lithium bis(trifluoroethyl)dithiocarbamate, Li(FDEDTC), as a chelating agent for determining Cd in urine. The use of isotope dilution technique provides the advantage of freedom from matrix effects; and, precision and accuracy of the analyses are not affected by incomplete recovery. Precision and accuracy of isotope ratio measurements at 10 ng level were evaluated; memory effect was investigated and the isotope dilution GC-MS method was validated by determining Cd in NIST urine reference material.

Experimental

The $^{106}$Cd-enriched cadmium oxide (79 atom% $^{106}$Cd, Oak Ridge National Laboratory) used as an internal standard for isotope dilution was dissolved in 2% HNO$_3$. Dilute solutions were prepared from this stock solution on a weight basis for isotope dilution experiments. The internal standard was calibrated by reverse isotope dilution GC-MS using the natural Cd primary standard.

One mL of the urine reference material was mixed separately with a known weight of standardized $^{106}$Cd internal standard solution. Urine samples were digested using HNO$_3$ + H$_2$O$_2$ mixture. The residue after digestion was dissolved in 2 mL of deionized water and undigested lipids were extracted prior to metal-chelate formation. A 20 mM solution of Li (FDEDTC) in deionized water was used to form Cd(FDEDTC)$_2$ chelate at pH 3 in an acetate buffer. The chelate was extracted with CH$_2$Cl$_2$ for GC-MS analysis.

Cd isotope ratios were measured in duplicate, by injecting 1 μL of the chelate solution and monitoring the isotopic cluster corresponding to the molecular ion. The sample was injected, on column, into a 10 m DB-1 bonded-phase fused silica capillary column. Data were obtained in the selected ion monitoring mode using a Finnigan MAT-8230 GC-MS system operated in the electron ionization mode and quantitation was achieved using the chromatographic peak area.

Results and Discussion

The 70 eV El mass spectrum of Cd(FDEDTC)$_2$ showed two groups of ions, molecular ion Cd(FDEDTC)$_2$$^+$ and fragment ion Cd(FDEDTC)$^+$ occurring at nominal m/z of 628 and 370, respectively. The most abundant isotopic group corresponding to the molecular ion was used throughout these experiments to achieve high sensitivity. This group consisted of nominal m/z 618, 620, 622, 623, 624, 625, 626 and 628, corresponding respectively to $^{106}$Cd, $^{108}$Cd, $^{110}$Cd, $^{111}$Cd, $^{112}$Cd, $^{113}$Cd, $^{114}$Cd and $^{116}$Cd.
The precision of the determination of isotope ratios was evaluated by injecting 1 µL of chelate solution containing about 10 ng of Cd. Replicate determinations were made on different days. Table 1 shows the results of precision evaluation. Overall precision values of 1 to 3% were obtained for the different isotope ratios, except for the m/z 618/626 and 620/626 ratios which gave a precision of 6 to 7% due to the low natural abundances of $^{105}$Cd (1.25 atom%) and $^{108}$Cd (0.89 atom%).

Memory effect was investigated by analyzing two synthetic mixtures differing in m/z 618/626 ratio by a factor of 10. A small memory effect was observed which can be minimized by optimum addition of the internal standard using a good experimental design.

The accuracy of the isotope dilution GC-MS method was validated by the quantitation of Cd in NIST freeze dried urine SRM-2670. Good agreement between the NIST certified values and experimentally determined results was obtained as shown in Table 2. This work has led to further studies with ICP-MS and TIMS for a definitive measure of the relative precision and accuracy of this isotope dilution chelation method.

**Conclusions**

Stable isotope dilution-gas chromatography-mass spectrometry using Li(FDIDTC) as a chelating agent can be used for Cd determination in urine at the 10 µg/L level with a high degree of precision and accuracy. There is a small memory effect which can be minimized by optimum addition of the internal standard solution.

<table>
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<tr>
<th>Isotope Ratio</th>
<th>Mean Value</th>
<th>Within-run Precision</th>
<th>Between-run Precision</th>
<th>Overall Precision</th>
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<td>1.8</td>
<td>6.6</td>
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<tr>
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<td>6.0</td>
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<tr>
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<td>2.3</td>
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<tr>
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<td>0.8</td>
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<tr>
<td>625/626</td>
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<td>0.9</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td>628/626</td>
<td>0.4161</td>
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<td>2.7</td>
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**Table 2**

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<th>Sample</th>
<th>NIST (µg/L)</th>
<th>Determined (µg/L)</th>
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</thead>
<tbody>
<tr>
<td>Urine SRM-2670</td>
<td>88 ± 3</td>
<td>94 ± 10</td>
</tr>
<tr>
<td>Diluted Urine SRM-2670</td>
<td>6.4 ± 0.3</td>
<td>9.3 ± 0.9</td>
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</tbody>
</table>
AN ENTROPY BASED DECONVOLUTION METHOD FOR OBTAINING PARENT MASSES WITH MIXTURES OF MULTIPLY-CHARGED IONS IN ESI-MS. Bruce Reinhold and Vernon Reinhold, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115.

Electrospray ionization of many protein and peptide samples produce mixtures of different molecular ions, each with a range of charge states. With glycoprotein samples, this can be attributed to differing glycotypes and glycoforms, (and other post-translational modifications during biosynthesis), which are extremely difficult to resolve chromatographically. The mass spectra of such materials can generate patterns of considerable complexity, making it a formidable challenge to extract parent masses.

This report describes an algorithm for the extraction of parent masses from ESI spectra consisting of mixtures of multiply-charged ions. The algorithm is based on the relative (level-2) entropy as a pattern recognition procedure. Entropy approaches have been used in a number of other applications such as clustering in image processing in astrophysical studies. Application of this algorithm to typical ESI-MS spectra has resulted in marked improvement of peak detection vs artifacts, a feature most valuable with mixtures of considerable complexity.

Some numerical band resolution enhancement techniques (Fourier deconvolution, Fourier differentiation) are illustrated in their application to poorly resolved peaks. These techniques amplify the midrange Fourier components relative to the low frequency components. This narrows peaks and thereby reduces artifacts due to 'accidental' overlap. The techniques also introduce a high frequency cutoff to reduce noise. These issues are illustrated with synthetic spectra (computer generated) and selected ESI spectra of glycopeptides.
An example of a typical complex ESI mass spectrum. The baseline does not exist and many of the peaks overlap.

Processing in Fourier Space

The idea is to increase the weight of the high frequencies and the importance of the high frequencies is the high frequency content of the data.

Spectral Deconvolution

Spectrum
Applications of an Improved Technique for the Transformation of the Electrospray Spectra of Proteins on to a True Molecular Mass Scale.

INTRODUCTION
In the form initially produced by the spectrometer, the electrospray spectra of protein mixtures are complex, each protein in the mixture being represented by a series of multiply charged ions. These ions occur with mass to charge ratio (m) given by:

\[ m = \frac{M+nH}{n} \]

where \( M \) is the molecular mass of the protein
and \( n \) is the number of charges on an ion, a series of consecutive integers.

A 15kDa protein typically produces about 10 peaks with a range of \( n \) from 10-20. Larger proteins, eg. albumins (~ 66,000) often have 20 or more peaks in the series.

To aid interpretation some means is needed for simplifying electrospray spectra, so that each component in a mixture of proteins is represented as a single peak on a true molecular mass scale. Previous methods (ref) suffer from increased baseline noise and tend to produce artefacts. The object of this paper is to describe a method of reliably transforming electrospray data which preserves resolution, directly gives the molecular mass, improves the signal to noise ratio, and above all is less prone to the generation of artefacts.

METHOD
The electrospray spectra of proteins are normally acquired by operating the data system as a multichannel analyser. Typically, several data channels are used to record each dalton along the mass/charge (m/z) ratio axis.

To transform the data into a true molecular mass spectrum, the multiply charged series are first identified and hence the number of charges associated with each peak can be assigned. Data system algorithms for carrying out this procedure, either automatically or interactively are based on the equation shown above. Once the number of charges on each peak has been assigned, every channel on the m/z axis is assigned a charge value. Each channel in the m/z spectrum is then transformed to a molecular mass scale using \( M=n(m-H) \), and the resulting data summed.

RESULTS
The data shown in Figure 1 was obtained from a mixture of normal human haemoglobin (alpha globin = 15126.4; beta globin = 15867.2) and horse heart myoglobin (16951.5). Figure 1(a) shows part of the original electrospray spectrum of the mixture with peaks from the 3 major components (A,C,D) plus a minor component (B). The data after peak detection is shown in Figure 1(b). Figure 1(c) shows the data after transformation. Note that minor Na & K adduct peaks associated with the alpha and beta globin peaks (A and B) are faithfully reproduced. Also note that even though the 15\(^+\) ion from normal beta globin (C15) is only partially resolved from the 16\(^+\) ion from myoglobin (D16), there are no significant artefacts in the transformed data.

To illustrate the more general applicability of the transformation technique to multiply charged ion series from bio-polymers, Figure 2(a) shows the negative ion spectrum of a 14 base oligonucleotide (calculated mass 4261.9) analysed in neutral solution. Note the dramatic way in which the relative intensities of the sodium adducts change with charge state. Figure 2(b) shows the data transformed onto a true molecular mass scale.

Fig. 1. (a) Part of the electrospray spectrum from a mixture of normal human haemoglobin and myoglobin, (b) after peak detection and (c) after transformation.

Fig. 2. (a) The negative ion electrospray spectrum of a 14 base oligonucleotide and (b) the data after transformation into a mass spectrum.
HIGH MOLECULAR WEIGHT FOURIER-TRANSFORM MASS SPECTROMETRY

Fourier-transform mass spectrometry (FTMS) has exciting attributes for the analysis of large molecules: ultrahigh resolution, high mass-measuring accuracy, nondestructive ion detection, simultaneous ion detection over a wide m/z range, and extensive capabilities for tandem mass spectrometry (MS/MS, MS"). Combined with electrospray ionization (ESI), which generates multiply charged ions, the mass range of FTMS has been extended to biomolecules as large as bovine albumin (MW 66,267). While spectra containing multiply charged ions of many masses present a difficult problem for charge state assignment on non-FT instruments, necessary for determination of m from m/z values, the resolving power demonstrated by FTMS allows unequivocal charge state assignment by simply counting the number of $^{13}$C isotope peaks within a single m/z unit, Figure 1 (1,2).

The atmospheric pressure ESI source has been coupled to FTMS using 5 stages of differential pumping, maintaining the analyzer at 10^-9 torr, and 3 rf-only quadrupoles. Sensitivity is enhanced by thermally cooling the ions prior to mass analysis, using long ion storage times (>1,000 s) or by using pulsed gas admission to the cell coincident with ion admission thus reducing the required cooling period to 100-200 s. Resolving powers in excess of 60,000 using broadband detection (all ions m/z >400 are simultaneously detected in 1.7 s) have been obtained for single scan spectra of cytochrome c isomers (MW 12,230-12,360), Figures 1 and 2, and for equine myoglobin (MW 16,950) with femtomole sensitivity. The average measured isotopic distribution for the 14+ - 17+ charge states of Figure 3 are denoted as circles superimposed on the theoretical isotopic distribution (Figure 4). Poor reproducibility of the isotopic abundances causes difficulty in matching these against calculated isotope distributions, which is necessary for identifying the most abundant isotope and for determining the isotopically-averaged MW.

ESI-FTMS has been demonstrated to be a valuable analytical tool in the structural characterization of large biomolecules. Mass measuring errors of less than 0.003% were achieved with an external m/z calibration, while an internal calibration using overlapping isotopic peaks of different charge gave mass measuring errors of less than 0.001%. Spectra of bovine ubiquitin (MW 8,565) have identified a low abundance impurity not previously resolved with quadrupole instruments, and MS/MS spectra using nozzle/skimmer dissociation and CAD confirm the charge state assignments for fragment ions reported from low resolution quadrupole spectra. Further MS" advantages can be gained by using the Hadamard transform [3] and remeasurement [4] techniques unique to FTMS.

Figure 1
Single scan spectrum of equine cytochrome c (MW 12,360):
• 9 femtomoles
• Avg. Res. = 78,000

Figure 2
Single scan spectrum of 1:1 mixture of porcine and chicken cytochrome c:
• ΔMW = 7 Da
• 4 femtomoles each
• Avg. Res. = 64,000

Figure 3
23 scans of 1:1 mixture of porcine and chicken cytochrome c:
• Improved isotope abund.
• Avg. Res. = 21,000

Figure 4
Measured ( ○ ) and theoretical isotopic distribution for the 14+ thru 17+ charge states of Figure 3 data.

* The isotope peak calculated to be the most abundant.
Electrospray Ionization on a Magnetic Sector Instrument with High Performance Array Detector

*Peter Dobberstein, Ulrich Giessmann, and Ernst Schroeder, Finnigan MAT GmbH, Barkhausenstr. 2, W-2800 Bremen 14, Germany

Data from a newly designed ESI source for magnetic sector instruments are presented. The source has 2 stages of differential pumping between the spray and the beam focusing region, keeping the pressure in the analyzer region below 10^{-7} Torr.

Gramicidin S was found to be an ideal substance for sensitivity measurements. The detection limit for full spectra at resolution 1,000 was determined by continuous infusion of 200 fmol/µl in MeOH/water at 1µl/min.

With the normal detector (slit + sem) a detection limit of 25 fmol was achieved. With the PATRIC™ array detector this was improved to 0.5 fmol (Fig. 1).

For the measurement of bovine insulin at a resolution of 8,000 (10% valley) signal averaging was applied and about 30 pmol have been consumed. With CsJ added as reference compound the accurate masses of the 4+ isotope cluster could be determined within 10 ppm (Fig. 2).
A full spectrum of myoglobin was acquired from less than 1 pmol using the normal detector (Fig. 3).

With the BIOMASS deconvolution algorithm the spectrum of multiply charged ions was transformed into a "molecular weight spectrum". The known molecular weight of myoglobin is found within ± 1 da (Fig. 4).

An example of a glycoprotein is ovalbumin (chicken egg). It generates relatively broad peaks, indicating the presence of at least 2 different components (Fig. 5).

Conclusion
ESI on a magnetic MS with array detector was demonstrated to have very high sensitivity and resolving power.
Electrospray ionization has expanded the role of mass spectrometry in the area of biochemistry through better determination of the molecular weights of peptides and small proteins. The greatly increased mass accuracy, relative to conventional methods such as gel electrophoresis, enables accurate determination of protein modifications. With careful measurement, average mass accuracies for proteins of 0.01% are being obtained with quadrupole and magnetic sector instruments. However, there are a number of protein modifications that result in small changes in mass that cannot be determined with 0.01% accuracy. For example, point modifications such as substitution of Asn for Leu, Glu for Lys, Asp for Asn, Glu for Gin, or a carboxy terminal OH for NH$_2$ all represent ca. a 1 u shift in mass. A 1 u difference in mass at MW 10,000 requires that mass measurement be better than 0.005%.

We have consistently obtained mass accuracies better than 0.003% (Table) for reasonably pure proteins to ca. 17,000 MW with an electrospray source interfaced to a ZAB-E double focusing magnetic sector instrument. For synthetic peptides between 5,000 and 10,000 u better than 0.002% mass accuracy has been consistently achieved. Accurate mass data were obtained by acquiring spectra of the peptide or protein with an internal reference mass standard, although preliminary work indicates good accuracies can be achieved running the sample and reference consecutively under identical conditions. The operating conditions for obtaining data were magnet scanning from 2,000 to 500 u at 20 s/dec using a resolution of between 1,200 and 2,000 (10% valley) and either PEG or PTMEG as an internal mass reference. From 5 to 25 scans of continuum time data were acquired into a single averaged file and the data were processed off-line on a VG OPUS workstation.

Consistent accurate mass data requires considerable manual intervention in either eliminating improperly centroided peaks or applying manipulations such as drawing baselines and peak smoothing. The difficulty in obtaining high accuracy is apparent when one realizes that for a 10,000 MW polymer, the +10 charge state molecular ion isotope cluster has a mass width of ca. 1 u and must be measured to 0.01 u to achieve 10 ppm accuracy. Because the isotope spread is ca. 1 u, resolution cannot be used to eliminate interferences (Figure 1). Figures 1 and 2 show the effect of resolution vs. mass. At MW ca. 5,000, 1,000 resolution gives the minimum peak width, whereas at MW 10,000, 2,000 resolution (10% valley) is necessary to achieve the minimum peak width of the isotope cluster. Interferences that cannot be removed with resolution will distort the mass measurement, but they can usually be identified by observing the peak symmetry and width.

Another factor that reduces mass accuracy is improper centroiding due to insufficient ion signal to adequately define the peak. This problem is most prevalent when sample limited or with samples that fail to give adequate signal by electrospray ionization. Sometimes good results can be achieved by 'eyeballing the centroid'.

It must be noted that there are many structural possibilities in proteins and modified proteins that will produce a 1 u mass shift. Thus, while increased mass accuracy adds confidence, used alone it can only eliminate possibilities that fall outside the mass accuracy window.

# ACCURATE MASS

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<tr>
<th>Peptide</th>
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<th>meas. MW</th>
<th>calc. MW</th>
<th>Δamu</th>
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</tr>
</tbody>
</table>

**Effect of Resolution on Bovine Insulin (×5) Ion**

**Fig. 1**

**Effect of Resolution on Lysozyme (×10) Ion**

**Fig. 2**
Electrospray Ionization on a Double Focusing Magnetic Sector Mass Spectrometer

R.T.Gallagher*, J.R.Chapman* and E.C.Barton†

*Kratos Analytical, Barton Dock Road, Manchester, UK
†Depart. of Chemistry, University of Cambridge, Cambridge, UK

Introduction

Electrospray ionisation is now regarded as an extremely powerful technique for the formation of intact ions from complex, high molecular weight species in solution. This technique is now coupled to a CONCEPT mass spectrometer and routinely shows the benefits of high resolution, high sensitivity mass spectrometry.

Interfacing

The electrospray source is mounted on a modified source housing door. The instrument operates at an accelerating potential of 4 kV with no requirement for discharge suppression on the additional two stage pumping system. All spectra shown here were recorded by spraying a 50:50 methanol:water mixture solution of samples at a concentration of 10 pm M via a tapered stainless steel needle of 0.12 mm I/D. A flow rate of 2.4 µl min \(^{-1}\) was used. The source housing pressure is \(10^{-6}\) to \(10^{\text{torr}}\) during normal operation.

Example: Bovine Albumin

The number of charges acquired in electrospray for a given molecular weight is affected by a number of factors such as sample chemistry and spraying conditions. If the number of charges is very high, the peaks due to adjacent charge states are very close in mass and therefore less well resolved.

Bovine albumin (M.Wt. 66,500) with 50 charges (m/z 1330.5) and 51 charges (m/z 1304.4) gives peaks only 26 amu apart. On the other hand, bovine albumin with 30 charges (m/z 2217.5) and 31 charges (m/z 2145.9) gives peaks over 71 amu apart. These lower charge, higher m/z ions can be confidently recorded with good sensitivity as this data shows.

The benefit of electrospray on high mass range sector mass spectrometers is illustrated again in the spectrum of bovine albumin dimer at a molecular weight of 133,000. This shows very highly charged ions which extend to over m/z 3000.
Resolution and Mass Accuracy

One advantage of sector instruments is high resolution for lower molecular weight compounds - i.e. individual isotopes may be resolved. The spacing of these isotope peaks allows the number of charges to be determined by inspection rather than by calculation. This is useful in direct mixture analysis.

Resolution is also important for mass assignment since poorly resolved multiplets will result in incorrect assignments. Molecular ion peaks at high molecular weight are already broad due to natural isotopic abundance so that a resolving power of 2,000 to 3,000, which can be achieved on a magnetic sector instrument with very little loss in sensitivity, is sufficient to reproduce the overall isotopic profile with relatively little broadening. Thus the use of such resolving power offers considerable benefits for routine electrospray. Table 1 shows that mass measurement precision is improved with an increase in resolving power. The authors are currently working on data processing techniques to maximise the accuracy of these measurements.

Higher Mass Samples

Additional full scan experiments suggest that a similar precision of mass measurement may be attained at higher molecular weights. Figure 4 shows part of the electrospray spectrum, also recorded using a CONCEPT I H instrument at a resolving power of 1000, of horse radish peroxidase, a glycoprotein that has a sugar content of approximately 20%. Mass measurement of the two most abundant components recorded in this scan, using the data from the 19*, 20*, 21* and 22* states in each case, gives molecular weight values of 42,343.2 ± 2.2 and 43,164.8 ± 3.2. These measurements correspond to a precision of ±0.005% and ±0.007% respectively (Table 2). These results are very promising, especially considering the improvements that might be expected at higher resolving power.

References

Table 1: Mass measurement precision versus resolving power

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<thead>
<tr>
<th>Compound</th>
<th>Resolution 1000</th>
<th>Resolution 1600</th>
<th>Resolution 2000</th>
<th>Resolution 2400</th>
<th>Resolution 3000</th>
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<tbody>
<tr>
<td>Bovine Insulin</td>
<td>±0.012</td>
<td>±0.005</td>
<td>±0.000</td>
<td>±0.008</td>
<td>±0.0035</td>
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<td>Hen Egg Lysozyme</td>
<td>±0.008</td>
<td>±0.0035</td>
<td>±0.000</td>
<td>±0.011</td>
<td>±0.010</td>
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<td>Horse Myoglobin</td>
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<td>±0.0035</td>
<td>±0.000</td>
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Table 2: Mass measurements from horse radish peroxidase

<table>
<thead>
<tr>
<th>Minor series</th>
<th>Major series</th>
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<tbody>
<tr>
<td>42343.2 ± 2.2</td>
<td>43164.8 ± 3.2</td>
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<tr>
<td>(±0.005%)</td>
<td>(±0.007%)</td>
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</table>

Figure 4: Spectrum of horse radish peroxidase
ELECTROSPRAY ON A MAGNETIC SECTOR MASS SPECTROMETER


Fisons Plc., VG Analytical, Floats Road, Wythenshawe, Manchester, M23 9LE, England.

Electrospray ionisation, by virtue of its propensity to generate multiply charged ions, has been used with quadrupole and ion trap mass spectrometers to analyse large biomolecules with molecular weights up to 100K Dalton.

An electrospray ion source has been designed for the AutoSpec double focusing high performance magnetic sector mass spectrometer (Fig 1). It incorporates two differentially pumped intermediate regions between the atmospheric pressure chamber and high vacuum. The intermediate regions employ a novel ion optical arrangement designed to optimise ion transmission whilst removing most of the neutral species. Ion acceleration subsequently takes place at high vacuum (typically $1 \times 10^{-5}$ mBar) in the source housing of the mass spectrometer, where ion/neutral collisions are sufficiently infrequent to allow good transmission of large, multiply charged ions (Figs 2 and 3). Fig 4 illustrates the transformation of raw electrospray mass spectra onto a molecular mass scale.

High resolution analyses can be carried out with ease on a high performance instrument such as the AutoSpec, and Fig 5 shows the quintuply charged ion envelope from bovine insulin (average MW 5733.6). This spectrum was obtained at a resolving power of 7000 (10% valley definition).

Fig 6 shows a comparison between high and low energy collision regimes for structural elucidation of a small peptide, methionine enkephalinamide (MW 572). Most of the observed product ions can be readily interpreted in terms of the structure of this peptide. The ion at m/z 498 in the B/E linked scan is characteristic of decompositions induced by high energy collisions.

FIGURE 1

FIGURE 2
Resolution and Mass Accuracy

One advantage of sector instruments is high resolution for lower molecular weight compounds—i.e., individual isotopes may be resolved. The spacing of these isotope peaks allows the number of charges to be determined by inspection rather than by calculation. This is useful in direct mixture analysis.

Resolution is also important for mass assignment since poorly resolved multiplets will result in incorrect assignments. Molecular ion peaks at high molecular weight are already broad due to natural isotopic abundance so that a resolving power of 2,000 to 3,000, which can be achieved on a magnetic sector instrument with very little loss in sensitivity, is sufficient to reproduce the overall isotopic profile with relatively little broadening. Thus the use of such resolving power offers considerable benefits for routine electrospray. Table 1 shows that mass measurement precision is improved with an increase in resolving power. The authors are currently working on data processing techniques to maximise the accuracy of these measurements.

Higher Mass Samples

Additional full scan experiments suggest that a similar precision of mass measurement may be attained at higher molecular weights. Figure 4 shows part of the electrospray spectrum, also recorded using a CONCEPT I H instrument at a resolving power of 1000, of horse radish peroxidase, a glycoprotein that has a sugar content of approximately 20%. Mass measurement of the two most abundant components recorded in this scan, using the data from the 19+, 20+, 21+ and 22+ states in each case, gives molecular weight values of 42,343.2 ± 2.2 and 43,164.8 ± 3.2. These measurements correspond to a precision of ±0.005% and ±0.007% respectively (Table 2). These results are very promising, especially considering the improvements that might be expected at higher resolving power.

Table 1: Mass measurement precision versus resolving power

<table>
<thead>
<tr>
<th>Compound</th>
<th>Precision of mass measurement in %</th>
<th>Observed molecular weight</th>
<th>Calculated molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine Insulin</td>
<td>±0.012 ±0.005 ±0.000 ±0.000</td>
<td>5734.0</td>
<td>5733.6</td>
</tr>
<tr>
<td>Hen Egg Lysozyme</td>
<td>±0.008 ±0.0035 ±0.000 ±0.000</td>
<td>14,305.2</td>
<td>14,305.2</td>
</tr>
<tr>
<td>Horse Myoglobin</td>
<td>±0.011 ±0.010 ±0.0035 ±0.000</td>
<td>16,950.9</td>
<td>16,950.5</td>
</tr>
</tbody>
</table>

Table 2: Mass measurements from horse radish peroxidase

<table>
<thead>
<tr>
<th>Mass (amu)</th>
<th>Minor series</th>
<th>Major series</th>
</tr>
</thead>
<tbody>
<tr>
<td>42,343.2</td>
<td>43,164.8</td>
<td></td>
</tr>
<tr>
<td>±0.005%</td>
<td>±0.007%</td>
<td></td>
</tr>
</tbody>
</table>

References

Figure 4: Spectrum of horse radish peroxidase
Electrospray ionisation, by virtue of its propensity to generate multiply charged ions, has been used with quadrupole and ion trap mass spectrometers to analyse large biomolecules with molecular weights up to 100K Dalton.

An electrospray ion source has been designed for the AutoSpec double focusing high performance magnetic sector mass spectrometer (Fig 1). It incorporates two differentially pumped intermediate regions between the atmospheric pressure chamber and high vacuum. The intermediate regions employ a novel ion optical arrangement designed to optimise ion transmission whilst removing most of the neutral species. Ion acceleration subsequently takes place at high vacuum (typically 1 x 10^-5 mBar) in the source housing of the mass spectrometer, where ion/neutral collisions are sufficiently infrequent to allow good transmission of large, multiply charged ions (Figs 2 and 3). Fig 4 illustrates the transformation of raw electrospray mass spectra onto a molecular mass scale.

High resolution analyses can be carried out with ease on a high performance instrument such as the AutoSpec, and Fig 5 shows the quintuply charged ion envelope from bovine insulin (average MW 5733.6). This spectrum was obtained at a resolving power of 7000 (10% valley definition).

Fig 6 shows a comparison between high and low energy collision regimes for structural elucidation of a small peptide, methionine enkephalinamide (MW 572). Most of the observed product ions can be readily interpreted in terms of the structure of this peptide. The ion at m/z 498 in the B/E linked scan is characteristic of decompositions induced by high energy collisions.

**FIGURE 1**

**FIGURE 2**
Recent developments in electrospray ionization have prompted widespread interest among mass spectrometrists studying large molecules. Most of these investigators use, and will continue to use, liquid secondary ion mass spectrometry (SIMS) ionization in their work. In many laboratories the same mass spectrometer will be used for both electrospray and liquid SIMS necessitating reconfiguration of the instrument when changing between the two ionization methods. Faced with this prospect, we decided to implement electrospray ionization on our triple quadrupole instrument (Nermag R30-10) so that we could easily switch between liquid SIMS and electrospray modes. The triple quadrupole instrument was previously modified to incorporate a cesium ion gun in which the cesium ion beam was coaxial with the sample probe and perpendicular to the quadrupole axis. To implement electrospray ionization, we chose the recently published design of Chowdhury, Katta, and Chait [1] which employs a heated metal capillary for desolvation.

**Experimental**

A diagram of the ion source is shown in Figure 1. The components are as follows: A. Hamilton 1705RN syringe with a Hamilton 80426 needle (25 gauge) driven by a Harvard Apparatus Model 11 syringe pump. The high voltage required to generate the spray is applied to the syringe needle. A Teflon insulating cap is placed over the syringe plunger handle to prevent discharge through the syringe plunger to the pump; B. Stainless steel counterelectrode disc insulated from the stainless steel capillary with Kel-F bushing; C. Upchurch Scientific P-640 Kel-F adapter, 1/4-28 thread to 10-32 Fingertight chromatography fitting sealed to flange with Teflon O-ring; D. 20 cm. x 0.062” o.d. 0.25 mm. i.d. 316 stainless steel tubing; not shown is heater wire (0.020” nichrome) in fiberglass sleeving wrapped on the capillary and an iron-constantan thermocouple to monitor capillary temperature; E. Kel-F bushing to align capillary end with skimmer. The bushing is mounted in a stainless steel plate mounted on two threaded standoffs; F. Skimmer cone with 0.5 mm. orifice (Vestec VT 1020A) located 3 mm. from end of capillary; G. Hemispherical electrode (radius 0.375”; center of radius at entrance of first ion source lens) which serves as the repeller for liquid SIMS operation. The skimmer cone and hemispherical electrode are electrically connected to the ion source repeller voltage supply (0-40 volts). H. Teflon washer (0.030”) which insulates the skimmer mounting plate from the housing. The mounting plate is attached with six 2-56 nylon screws; I. Standard lenses of the Nermag R30-10 FAB ion source; J. First quadrupole mass analyzer; K. Cesium ion gun (Antek) mounted to FAB ion source block; L. Nermag FAB probe (shown retracted from ion source); M. Upchurch scientific F100 Kel-F Fingertight chromatography fitting used with 18 gauge copper wire in 1/16” o.d. Teflon tubing to provide electrical feedthroughs for heater and thermocouple wires (one shown of four); N. Conflat type flanges 2.75” o.d. mounted with Viton O-ring in place of copper gasket to maintain constant spacing; O. Conflat type flanges, 4.5” o.d. mounted with Viton O-ring; P. Pumping port connected via butterfly valve to cold finger and mechanical pumps.

For electrospray analysis the samples were dissolved in 47:47:6 (v/v/v) water/methanol/acetic acid and delivered to the needle at a rate of 0.5-1.0 μL/min. Typical operating voltages were: needle, 4.4 kV; capillary and counterelectrode plate, 250 V; and skimmer/repeller, 30 V. The capillary was maintained at a temperature of 90-100°C.

For liquid SIMS analysis the samples were applied in 5% acetic acid or 0.1% trifluoroacetic acid to a film of thioglycerol onto the end of a gold plated 2 mm. dia stainless steel probe tip. Cesium ions (7 kV energy) were used to desorb sample ions.

**Results**

The combination ion source gave comparable performance in liquid SIMS mode to the standard Nermag FAB ion source indicating that the hemispherical repeller was an adequate
substitute for the curved metal strip repeller on the standard FAB ion source. Some results of sample analysis in electrospray mode are shown in figures 2 and 3. Figure 2 shows results obtained for equine myoglobin; the measured molecular weight \((16,944 \pm 5)\) was within 0.037% of the calculated molecular weight \((16,950.5)\). The accuracy of these measurements is limited by the acquisition of nominal mass data and could likely be improved by acquisition of "profile" data and measurement of mass to charge ratios to tenths of a unit. Figure 3 shows data obtained on bovine serum albumin, the largest protein examined with this ion source. The measured molecular weight \((66,541 \pm 39)\) was within 0.4% of the calculated mass \((66,267)\), similar to results reported previously [1,2].

Conclusion

A combination electrospray-liquid SIMS ion source has been constructed which allows use of both modes of ionization without the need for physical reconfiguration of the instrument. The electrospray data obtained on samples of bovine insulin (mol. wt. 5,733.6; error 0.034%), equine myoglobin (mol. wt. 16,950.5, error 0.037%) and bovine serum albumin (mol. wt. 66,267; error 0.4%) are comparable to previously published data. The detection limit for bovine insulin was less than 8.32 fmol of material for a full scan. The electrospray source also performed satisfactorily on a second tandem quadrupole instrument which required a longer desolvation capillary indicating that the design should be adaptable to a variety of instruments.

References

ANALYSIS OF MIXTURES OF CLOSELY RELATED FORMS OF BOVINE TRYPsin BY ELECTROSpray IONIZATION MASS SPECTROMETRY. USE OF CHARGE STATE DISTRIBUTIONS TO RESOLVE IONS OF THE DIFFERENT FORMS. S.K. Chowdhury and B.T. Chait, The Rockefeller University, New York, N.Y. 10021

Electrospray ionization (ESI) mass spectrometry has been utilized for the analysis of a commercial sample of bovine trypsin (Sigma T8642). Commercial active bovine trypsin normally contains a mixture of three different forms of the enzyme, namely β-, α-, and ψ-trypsin (1). β-trypsin is a single chain enzyme, resulting from the cleavage of the Lys(6) - Ile(7) bond of trypsinogen with the release of the hexapeptide, VDDDDK. The calculated MM of bovine β-trypsin is 23293.3 u. α-trypsin originates from the additional hydrolysis at Lys(131) and, therefore, has a MM 18 u higher than the β-form. ψ-trypsin refers to a form with an additional split at Lys(176) and its MM is 18 u higher than the α-enzyme (Scheme I). Peak broadening caused by the natural isotopic abundance of atoms in the protein molecules results in overlap of the ion peaks from the different trypsin forms. Therefore, accurate determination of the molecular masses of these trypsins in a mixture is not straightforward, particularly when one component is present in small amount relative to the others. Even when various forms are present in nearly equal amounts, a resolution of approximately 1300 would be required to resolve ions from the different forms, a resolution higher than is frequently used with quadrupole mass analyzers coupled to an ESI source. We describe here a method whereby the closely related forms of trypsin in a mixture can be resolved in the ESI mass spectrum according to the different number of charges acquired by the different forms, allowing the accurate determination of their masses.

The electrospray ionization mass spectrum of bovine trypsinogen and bovine trypsin are shown in Fig. 1 and 2 respectively obtained with the help of an ESI mass spectrometer constructed at the authors’ laboratory (2). A single ion intensity distribution of charged states is observed in the mass spectrum of trypsin (Fig. 2). One distribution centers around the 18+ ion and the other around the 14+ ion. 18+ and 14+ represent ions with 18 and 14 protons respectively attached to the trypsin molecule. The calculation of molecular mass (MM) from the observed m/z values of ions designated as 20+ to 12+ indicates the presence of three different proteins (Table 1). The MM obtained from the 12+ to 15+ ions is 23295.7 ± 1.8, that from the 17+ to 20+ ions is 23324.9 ± 2.3, and that from the 16+ ion is 23309.6 u. Ions with intermediate charge states (15+, 16+, and 17+) may have unresolved contributions from the proteins producing the two flanking ion distributions. The multiple resolved ion components observed with the 12+ to 15+ ions arise from the attachment(s) of sulfuric acid and/or phosphoric acid molecule(s) to the protein ions (3). These acids are formed in the acidic spray solution from the sulfate/phosphate impurities present in the protein sample.

The observed MM of 23295.7 u closely agrees to that calculated for β-trypsin, the value 23249.4 corresponds to ψ-trypsin, and the intermediate value, 23309.6 u appears to be in agreement with the calculated MM of α-trypsin. It is expected that ions arising from α-trypsin will have higher charge states than those arising from β-trypsin because it has an additional free NH₂-group (from the internal cleavage) and because its less constrained structure allows it to acquire and hold on to a higher number of charges (4). Similarly, it is expected that ions arising from ψ-trypsin will acquire a higher number of charges than both α and β-trypsin. The observed electrospray ionization charge state distributions of the bovine trypsin isofoms (Fig 2) confirm these expectations. The results described above are of practical importance because the different trypsin forms produce ions with different charge state distributions and therefore, yield ions in different regions of the mass spectrum. Thus, an accurate determination of the molecular masses of the three closely related different trypsin forms that differ in MM by only 18 u is obtained. We predict that this method for analyzing closely related forms of proteins will be generally applicable to other cases where the different forms adopt different higher order structures.
REFERENCES.

Table 1. Observed and calculated molecular masses of trypsinogen and different trypsin forms.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Observed MM(u)</th>
<th>Charge States</th>
<th>Calculated MM(u)</th>
<th>ΔM (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>bovine trypsinogen</td>
<td>23981.6 ± 2.0</td>
<td>13-16</td>
<td>23981.1</td>
<td>+ 20</td>
</tr>
<tr>
<td>bovine trypsin</td>
<td>23293.3 ± 1.8</td>
<td>12-14</td>
<td>23293.3(β)</td>
<td>+ 103</td>
</tr>
<tr>
<td></td>
<td>23311.3(α)</td>
<td>16</td>
<td>23311.3(α)</td>
<td>- 73</td>
</tr>
<tr>
<td></td>
<td>23324.9 ± 2.3</td>
<td>17-20</td>
<td>23324.9(ψ)</td>
<td>- 193</td>
</tr>
</tbody>
</table>

a: Difference between the observed (column 2) and the calculated molecular mass (column 4).

![Fig. 1](image1.png)

**Scheme I**

Trypsinogen

\[ \text{Cleavage of N-terminal hexapeptide (VDDDDK)} \]

β-trypsin

\[ \text{Cleavage at Lys - 131} \]

α-trypsin

\[ \text{Cleavage at Lys - 176} \]

ψ-trypsin

![Fig. 2](image2.png)
ELECTROSPRAY IONIZATION MASS SPECTROMETRY OF SYNTHETIC PEPTIDES AND PROTEINS. COMPARISON WITH $^{252}\text{Cf}$ PLASMA DESORPTION MASS SPECTROMETRY.

The Rockefeller University, New York, NY 10021.

Synthetic peptides and proteins are becoming of considerable use in diverse areas of biological science, immunology and medicine. We have shown previously (1) that $^{252}\text{Cf}$ plasma desorption mass spectrometry (POMS) is a powerful analytical tool for the analysis of synthetic peptides and proteins. Here we report the use of electrospray ionization (ESI) mass spectrometry for the analysis of such compounds. The study is based on the detailed analysis of more than 300 synthetic peptides and proteins in the authors' laboratory.

The ESI mass spectrometer used for the study has been described previously (2). Because the sequence of the target peptide is known, the determination of the molecular weight (MW) provides a rapid verification of the primary structure of the peptide. Disagreement between the observed and the calculated MW indicates a possible modification of the peptide and provides clues regarding the nature of the modification. Another important use of the technique is the rapid assessment of the homogeneity of the target peptide. We find that ESI mass spectrometry provides an enormously useful complementary tool to the more commonly employed HPLC analysis. Fig. 1 shows an electrospray ionization mass spectrum of Asn$^{12}$ transforming growth factor-α. In addition to the ions corresponding to the correct MW, we also observe a by-product 58.2u higher than the target compound. High resolution HPLC analysis of the above protein produced a single, symmetric peak (Fig. 1a). Another example is given in Fig. 1b which shows an ESI mass spectrum of endothelin. The spectrum shows peaks corresponding to a peptide 41.8u higher than the calculated MW of endothelin. One probable cause for this mass discrepancy could be acetylation.

We also make a detailed comparative evaluation of ESI with $^{252}\text{Cf}$ PDMS for the analysis of synthetic peptides and proteins. Factors such as mass accuracy, speed, sensitivity, resolution, ability to handle mixtures, universality, and ease of interpretation are compared (Table 1). Each of the techniques was found to have particular merits and drawbacks. Fig. 2a shows an ESI spectrum of an analog of mast cell degranulating peptide which was difficult to interpret. On the other hand, $^{252}\text{Cf}$ PDMS spectrum of the same peptide (Fig. 2b) was much easier to interpret. Therefore, PDMS can be used to analyze mixtures of synthetic peptides and proteins.

The intensity of the peptide and protein ions in the mass spectra is strongly dependent on the stability of the spray, which in turn, depends on the purity of the analyte. Presence of strong acids such as HCl, H$_2$SO$_4$, TFA etc. above 1% and/or salt concentration above 10mM perturb the stability of the spray and reduce the quality of the spectra. We use ESI routinely to analyze synthetic peptides and proteins because of its higher speed and use PDMS where ESI spectra are difficult to interpret and/or obtain.

References.

2. HOMOGENEITY OF FINAL PRODUCT

Frequently, HPLC analysis yields a single peak from mixtures of closely related final products.

Fig. 1a

In addition to the target compound, a by-product is detected with a MM 58.2 u higher than the expected MM.

Table 1.

<table>
<thead>
<tr>
<th>ESI</th>
<th>PDMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SENSITIVITY</td>
<td>1 - 30 pmol</td>
</tr>
<tr>
<td>MASS ACCURACY</td>
<td>0.01 - 0.03 %</td>
</tr>
<tr>
<td>RESOLUTION</td>
<td>500 - 1000</td>
</tr>
<tr>
<td>SPEED (run/sample)</td>
<td>5 - 10</td>
</tr>
<tr>
<td>Ease of Interpretation</td>
<td>Usually easy</td>
</tr>
</tbody>
</table>

Table 1. COMPARISON OF ESI WITH PDMS SPECTRA

3. UNWANTED SIDE-PRODUCTS

Modification detected
Mass discrepancy = 41.8 u
Therefore, peptide is probably acetylated.

Fig. 1b

For synthetic peptides, however, acetylation is rarely an important factor because usually 1 amine is available.

** Approx 1 in 10 samples is more difficult

252c PDMS SPECTRUM OF MAST CELL DEGRANULATING Peptide

Meas. MM = 1953.5
Calc. MM = 1953.2

Fig. 2a

IKCNCKPH1CRKICGKN-NH2

251
INTRODUCTION

Electrospray ionization (ESI) mass spectrometry and matrix assisted laser desorption time-of-flight mass spectrometry (LD-MS) have shown great promise in the analysis of large biomolecules with molecular weight greater than 5000 amu. While FAB mapping of peptides is effective, particularly in the range below 5000 amu, we were interested in using ESI to analyze proteins and protein fragment mixtures above this mass range. Two ESI sample introduction techniques were used to examine clostripain digests of Ribonuclease A: Direct Infusion and LC/ESI. We have also used LD-MS for the construction of SEQUENCE-ORDERED peptide maps using clostripain digests of modified ribonuclease A.

EXPERIMENTAL

Clostripain digests were performed in 10 mM Tris/Cl, 1 mM CaCl$_2$, 2.5 mM DTT, pH 7.5, with Clostripetidase B (Promega) at an enzyme:substrate ratio of 1:100 (w/w). Approximately 1.36 mg of reduced pyridylethyl RNase A in 680 µL of the above buffer and 13.6 µL of activated enzyme solution were mixed. Aliquots were removed at 0, 1, 2, 4, 8, 16, 32, 51, 107, 151 minutes and at 23 hours.

The electrospray analyses were performed on a Finnigan MAT TSQ70 mass spectrometer fitted with a Finnigan electrospray source. All spectra were obtained in the positive ion mode using Q3. Deconvolution of the resulting spectra was done on a DECStation 2100 using the Finnigan BioMass Deconvolution program.

Direct Infusion was done using a drying gas temperature of 60°C and a solution flow rate of 2.25 µl/min. The digest aliquots were dissolved in 57.5% methanol/37.5% water/5% acetic acid to give a final concentration of 10 pmol/µl. The run times were 5 minutes with a total sample consumption of 125 pmols.

LC/ESI utilized a nitrogen drying gas temperature of 80°C and a nitrogen sheath gas pressure of 45 psi. Isopropanol was used as the sheath liquid with a flow rate of 2.25 µl/min.

LC was performed on a reverse phase Sherisorb OSD2 C$_{18}$ microcapillary column (250 µm ID x 21 cm) with a 20 cm transfer line (50 µm ID) connecting the column and the TSQ70. Two HPLC pumps were used with a splitter that gave a split ratio of 45:1 at a flow rate of 2 µl/min through the column. The mobile phase was acetonitrile/water/0.1% TFA. Total run time was 40 minutes using a linear gradient from 3-90% acetonitrile over 30 minutes and holding at 90% acetonitrile for an additional 10 minutes.

The digest aliquots were dissolved in water and 40 pmols were injected onto the column using a Rheodyne 8125 injection valve with a 5 µl loop. The mass spectrometer was used as the detector for the LC separations.

RESULTS

The Direct Infusion ESI deconvoluted spectrum of the 1 minute clostripain digest is shown in Figure 1. The deconvoluted spectrum of the 1 minute clostripain digest with LC/ESI, Figure 2, is averaged over the LC run after the injection peak elutes.

Peak detection is not as efficient using Direct Infusion for the analysis of low concentration components in a complex protein digest. The ratios of the peak intensities for both methods show the same relative trends. The largest peak for the 1 minute digest spectra for both methods is the molecular ion of the reactant protein (m/z 14531.5).

Using selected ion chromatograms to isolate the elution time of each fragment for the LC/ESI spectrum allowed identification of each peak present in the digest. In all, 15 out of 15 peptide fragments were found using LC/ESI while only 10 out of 15 were found by Direct Infusion.

Comparison of the LC/ESI spectra for the 1 minute clostripain digest with LD data shows differing peak intensity ratios than those obtained with either ESI method. The mass accuracy of the LC/ESI data is equivalent to that obtained with LD-MS (Table 1). For LD/MS 13 out of 15 possible peptides were identified.
CONCLUSIONS

LC/ESI, in contrast to Direct Infusion, is the method of choice in analyzing concentration components in complex mixtures. Not all fragment peaks were identified using Direct Infusion ESI. Both LD-MS and LC/ESI MS give better results for fragment peak detection and subsequent digest analysis. The mass accuracy for both LD-MS and LC/ESI MS was, in general, 0.02% or better.

REFERENCES


ACKNOWLEDGEMENTS

The authors would like to thank the NIH (Grant Number GM43783-02) for its support, Robert Reiser (DuPont) for providing the C18 microcapillary LC column and John Cottrell (Finnigan MAT) for help in obtaining the LD data.

<table>
<thead>
<tr>
<th>Peak Label</th>
<th>Predicted Fragment</th>
<th>Calc (M+H)+</th>
<th>Laser Desorption Data &amp; Error</th>
<th>Calc M+H+</th>
<th>Electrospray Data &amp; Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1-10</td>
<td>1151.3</td>
<td>1151.1</td>
<td>1150.3</td>
<td>1150.6</td>
</tr>
<tr>
<td>D</td>
<td>11-33</td>
<td>2657.9</td>
<td>2657.9</td>
<td>2656.9</td>
<td>2656.9</td>
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<tr>
<td>C</td>
<td>14-29</td>
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<td>745.0</td>
<td>745.7</td>
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<tr>
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<td>4582.0</td>
<td>4583.7</td>
<td>4581.0</td>
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<tr>
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<td>6325.2</td>
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<td>4518.0</td>
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<td>9953.3</td>
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<td>10762.3</td>
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<td>10760.2</td>
<td>10759.7</td>
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<td>11-124</td>
<td>13402.9</td>
<td>13402.9</td>
<td>13399.1</td>
<td>13402.9</td>
</tr>
</tbody>
</table>

TABLE 1

COMPARISON OF LD-MS AND LC/ESI FRAGMENT ION MOLECULAR WEIGHT
Formation of charged droplets in electrospray

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Flow patterns which can be observed in nature have to satisfy the equations of hydrodynamics and, in addition, must be stable with respect to small perturbations. If this statement were not true, probably, electrospray would not exist at all. Instead of spraying, arbitrarily long thin liquid jets could be drawn from the high voltage capillary, and these liquid satisfy the hydrodynamic balance conditions which can be compressed in the form:

\[ \frac{2 \rho \rho_0^2}{\pi^3 R^3} - \frac{\varepsilon_0 V_0^2}{R \ln^2 R} + \gamma = 0 \]  

where \( R, Q, \rho, V_0, \) and \( \gamma \) are the radius of the jet, the flow rate, the density of the liquid, the capillary voltage, and the surface tension, respectively.

However, there are small perturbations, with respect to which liquid jets are unstable, which grow in amplitude until the jet will disrupt into droplets. The disintegration process will be dominated by the fastest growing unstable mode also called 'the most dangerous unstable mode' [1]. Spatial distribution of the small perturbations are described in terms of their Fourier components (modes) characterized by the wave number, \( k \). The temporal evolution of the modes is given by multiplicative factors of the form: \( \exp[-i \omega(k)t] \) where \( \omega \) is the complex frequency. Whenever the imaginary part of the frequency is positive, that mode will grow exponentially in time. The most dangerous mode represents the maximum of the (positive) imaginary part of the frequency as a function of the wave number. Dispersion equation for an electrified liquid jet has the form [2]:

\[ \omega^2 = \frac{\gamma}{\rho R^3} \left[ (k^2 - 1) - \frac{\varepsilon_0 V_0^2}{\gamma R \ln^2 R} \frac{x K_1(x)}{K_0(x)} \right] \frac{x I_3(x)}{I_2(x)} \]  

where \( x = kR \) and \( I_0, I_1, K_0, K_1 \) are modified Bessel functions in the standard notation.

In this model the size of droplets into which unstable electrified jets disrupt is determined by the wavenumber of the most rapidly growing unstable mode characterized by the wave number \( k' \). This wavenumber can be found where the imaginary part of the frequency is maximal (and positive). The most probable volume of droplets is approximated by a cylindrical volume of the radius \( R \) and of the length \( 2\pi/k' \). Thus, the most probable droplet radius:

\[ \langle \tau \rangle = R \left( \frac{3\pi}{2k'^2 R} \right)^{1/3} \]  

The general numerical procedure is the following: determine the jet radius at a given flow rate and capillary voltage from Eq. 1. Calculate the dispersion equation with the known radius and find the maximum of \( \text{Im}(\omega) \) as a function of \( k \) from the Eq. 2.

\[ \text{Present address: Department of Chemistry, MIT, Cambridge MA 02139} \]
Figure 1 Droplet radii compared for water and methanol as a function of the capillary voltage.

Figure 2 Droplet radii for water and methanol as a function of the flow rate.

References:
ESI/MS OF PROTEINS: WHY MORE CHARGES THAN EXPECTED?

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Electrospray mass spectra for peptides and proteins consist of multiply charged molecular ions each representing a charge state or m/z. A review of ESIMS analysis of peptides and proteins indicates that 80% of peptides and proteins analyzed in the positive ion mode have charge states equal to or less than the number of basic groups belonging to the expected "basic amino acid" residues Arg, Lys, His and the α-NH₂ group of the N-terminal residue. However, the other 20% of peptides and proteins were indicated to have unexpected charge states in excess of the "conventional" basic groups (Arg, Lys, His, & α-NH₂) present in the polymer. The extra proton acceptor sites on these peptides and proteins that appear to function in the ESI process have not been clearly defined. A question that arises is: Are there other amino acid residues with a side chain groups that may act similarly to the groups offered by the more conventional basic amino acid residues (Arg, Lys, His, & α-NH₂) present in the polymer? Amino acid proton affinity measurement studies involving the unimolecular decomposition of FAB-MS generated protonated amino acid dimers place glutamine high in the list with the basic residues. ESIMS studies on some glutamine containing proteins showing extra charge states such as human growth hormone (hGH) has led to the suggestion that glutamine (Q) residues may be involved.

In order to test the possible role of glutamine as a proton acceptor in the ESI process, we focused on a simple glutamine containing peptide case that was brought to our attention by the study of Lee et al. on the LC/ESIMS on a tryptic digest of biosynthetic bovine growth hormone (bGH) and our work on the LC/ESIMS on a tryptic digest of biosynthetic human growth hormone (hGH). Human GH and bGH are proteins that have amino acid sequences that are 70% identical. In both studies the last tryptic peptide to elute from C18 RP-HPLC columns was a very hydrophobic peptide identified as the respective T9 peptides of both species of recombinant GH. Interestingly, the bovine GH T9 gave an "unexpected" +3 charge state for its sequence while human T9 gave only the expected +2 charge state upon LC/ESIMS analysis. The T9 peptides from the two species differ in only three out of eighteen amino acid positions. The difference in the observed charge states for these two closely related peptides suggested that minor sequence differences may influence the charge state of a peptide ion produced by the ESI process.

Our strategy was to use a synthetic peptide analog approach in an attempt to map determinants for the extra charge state observed in the ESI/MS analysis of the bGH T9 peptide that showed the extra charge state previously seen.

We synthesized derivatives of the bGH T9 peptide using micro-methodology suitable for subsequent ESIMS analysis and noted which derivatives gave "unexpected" or "extra" charge states.

ESIMS analysis of several synthetic peptide derivatives shown in Table 1 led to the conclusion that the first glutamine in the structure was a proton acceptor. Figure 1 shows the ESIMS mass spectra for four "critical substitution" derivatives residue. Note that only the top structure containing two glutamines and the bottom structure having only the first glutamine in place show (M+3H) ions, the "extra" or "unexpected" charge state species.

We conclude with the following points:

1. The first glutamine (Q) of the bGH T9 peptide is "unique". Of the two glutamines present, the first alone acted as a proton acceptor. This indicates that glutaminyl residues are unequal in demonstrating this property. This result suggests that the microenvironment around the proton acceptor glutamine is important when the peptide is subjected to the ESI process.

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2. It is of utmost interest to note that prolactin (PRL), a protein hormone homologous to growth hormone (GH), which belongs to the GH-PRL family (these protein hormones are thought to be derived from a common ancestral gene) has a GH T9 homologous region. Comparison of the amino acid sequences of the T9 homologous regions for growth hormones and prolactins from several different species indicated that there are Arg (R) substitutions in most of the prolactins for the amino acid position corresponding to the unique glutamine proton acceptor in the homologous GH’s. Furthermore, within the prolactin family the mouse PRL has a glutamine substitution in this basic residue site.

3. This comparative sequence information coupled with our study has led us to the realization and the hypothesis that “unique” glutaminy1 residues detectable by ESIMS analysis may function as proton acceptors and mimic the more conventional basic residues (R, K, & H) in biologically functioning native proteins.

4. Protein chemists should consider glutamine as a “conservative” substitution for a basic amino acid in “some cases”. ESIMS analysis of synthetic variants of the glutamine containing peptide regions in question should aid in the identification of these “unique” proton acceptor glutamines.


Table 1

<table>
<thead>
<tr>
<th>Synthetic Peptide Derivative Summary</th>
<th>Extra Charge State</th>
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</thead>
<tbody>
<tr>
<td>ISSLIGGLQPLFSLR</td>
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<tr>
<td>LLSLIGGLQPLFSLR</td>
<td>YES</td>
</tr>
<tr>
<td>IQSLIGGLQPLFSLR</td>
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<tr>
<td>GQSLIGGLQPLFSLR</td>
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<tr>
<td>SGLIGGLQPLFSLR</td>
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<tr>
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<td>WEAK</td>
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<tr>
<td>LLSLIGGLQPLFSLR</td>
<td>YES</td>
</tr>
</tbody>
</table>

Fig. 1 Legend: ESIMS Spectra of “critical” residue substitutions. The sequence of the peptide is presented on the spectrum. The numbers in the +3 column represent the theoretical m/z for the “extra” charge state. The numbers in the +2 column represent the expected charge state m/z. Upper numbers are for the formylated Trp (W) peptide derivative. Formylated and unmodified peptides were analyzed as mixtures.

This work was supported by a grant from the NIH, Grant # 1 ROM1 GM43783-01.
A Comparison of In Source Fragmentation of Peptides vs. CID Products as Observed During Electrospray Ionization Mass Spectrometry
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The design and implementation of electrospray ionization (ESI) on magnetic sector mass spectrometers has been well documented. The principal advantage sector mass spectrometers offer are improved resolution and mass assignments for both "normal" and collision induced dissociation (CID) modes of analysis. The ability to obtain high resolution product ions derived from the collision of multiply charged precursor ions would enable product ion charge states and sequence determinations to be made much more reliably.

We have generated the product ion spectra for several peptides and small proteins at sufficient resolving power to identify product ion charge states based on the spacing of isotope peaks for doubly, triply and quadruply charged precursors. The implementation of ESI on a high performance JEOL SX102 magnetic sector mass spectrometer has also enabled us to acquire CID spectra of these compounds in two modes; (a) at low energy (via capillary/skimmer induced dissociation) and (b) at high energy (via constant B/E linked scanning techniques. The ability to effect fragmentation in both these regions has contributed greatly to the reliable interpretation of CID spectra. The CID generated via capillary/skimmer induced dissociation and at high energy (in the first field free region following the ion source) enabled complete sequence identifications to be made for bradykinin and renin substrate, which, under normal ESI operating conditions, produced exclusively the doubly and triply charged states, respectively. The in-source fragmentation of renin produced a series of a, b, and y ions, giving partial sequence information from positions 6-14 in the molecule. Only with the aid of collision induced dissociation at high energy was it possible to generate product ions which allowed for isomeric amino acids to be differentiated (e.g., leucine and isoleucine) and N-terminal sequence specific ions which helped elucidate the primary sequence of the molecule.

The ability to obtain useful sequence information when handling larger molecular weight peptides, though, is limited. We investigated the ability to fragment glucagon "in-source." Under normal ESI conditions (i.e., a capillary/skimmer 1 potential difference = 30V), glucagon exists primarily as the stable 3+ charged ion. Increasing the potential difference between capillary and skimmer 1 to 100V resulted only in a shift in the most abundant charge state (i.e., the 2+ ion). However, the ability to obtain B/E linked scan high energy CID data on the stable 3+ charge state for glucagon proved much more valuable. In the analysis of this peptide at high energy, a wealth of 2+ charge state product ions were observed. The charge state of these product ions were determined by operating the sector mass spectrometer at a nominal resolution of 1500, sufficient to partially resolve the isotope peaks of the respective product ions.

It is clear that alone, neither capillary/skimmer induced dissociation or high energy CID spectra contained sufficient product ion information to permit the complete sequence determination of these peptides. However, the combination of the two sets of data, do appear to provide sufficient sequence information to readily deduce primary sequences of low to medium molecular weight peptides.

The usefulness of this methodology was thus extended to the characterization of larger biomolecules. Therefore, we investigated the utility of generating sequence information by both capillary/skimmer induced dissociation and high energy CID for a model protein, amylin (MW=3920). The ESI mass spectrum of this protein is shown in Figure 1. The resolution of the mass spectrometer was set to 4000 in order to resolve sufficiently the spacing of isotope peaks and establish a charge state for the major ion in the spectrum. Capillary/skimmer induced dissociation did not produce any significant fragmentation. The spectrum generated at a capillary/skimmer
potential difference of 100V resulted only in a charged stripped mass spectrum, producing the 3+ charged state ion almost exclusively. The B/E linked scan high energy spectrum, on the other hand, provided a significant amount of structurally useful sequence information (as shown in Figure 2). The charge state of these product ions was obtained by operating at a resolution sufficient to partially resolve the isotope peaks. Interestingly, the major ions produced in this product ion analysis were of the type b3+ and y3+. We are currently investigating the use of other collision gases which might be used to produce more high energy-like fragments.

Electrospray Mass Spectrum of Amylin

B/E Linked Scan Mass Spectrum of Amylin (4+ Chg. State)

50% Attenuation using Helium
Sample Purification within A Mass Spectrometer: Enhancing The Quality of Protein Mass Spectra by Ion Kinetic Energy Filtering

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The presence of some non-protein substances, especially some polar polymers, in a protein solution has often been a nuisance to the electrospray or ionspray (pneumatically assisted electrospray) analysis, as they produce their own spectra with high sensitivities and wide mass distributions that sometimes overshadow or overlap the weak protein signals. These interfering background signals not only complicate the data analysis, but also compromise the accuracy of protein mass measurement by overlapping the protein peaks. In some cases these contaminants still remain even after a sample has gone through HPLC or dialysis purification. Additional sample clean-ups may not be warranted, as the small amount of the remaining contaminants is usually harmless to the ordinary biochemical research. We report here a new method for the removal of the concomitant non-protein ions within a quadrupole mass spectrometer without additional sample purification.

Ion kinetic energy profile analysis (using the Sciex API III MS) has revealed that non-protein ions, such as polymer ions due to adducts formation, in general have less kinetic energy than the heavier protein ions. As shown in Fig. 1, the retarding potential required to stop papain ions (23,422 Da, 14+ - 19+) is a few higher than the one needed to stop polypropylglycol ions (200 - 2,400 Da, 1+ - 2+). This kinetic energy difference may have resulted from the electrospraying and the atmospheric pressure sampling processes. First, the heavy protein ions may have gained more momentum (thus more kinetic energy) than the light non-protein ions during the supersonic free-jet expansion in the atmosphere/vacuum interface region. Second, the multi-charged protein ions may have retained more kinetic energy than the usually single- or double-charged polymer ions during their voltage-driven migration in the atmosphere. Third, during the supersonic expansion between the electrically biased sampling orifice and quadrupole guidance lenses, the multi-charged protein ions may have been accelerated more than the less-charged polymer ions. The observed effect is probably due to the combination of all three factors.

Taking advantage of this kinetic energy difference, these polymeric contaminant ions can be prevented from entering the quadrupole mass analyzer by putting on it a bias voltage (Fig. 2). The maximum bias voltage that can be applied without severely affecting the protein ions depends on the protein mass and charge state. Usually, the higher the protein mass, the higher the bias voltage it tolerates, and the more complete the contaminant removal. The clean protein ions from such kinetic energy filtering not only produce much cleaner mass spectra, but also increase spectral resolution due to the reduction of the background signals and the slowing down of the protein ions. In some dramatic cases, the sample analyses were not possible without kinetic energy filtering. For example, in a sample of chemically modified cathepsin B (28,762 Da) the protein signals were invisible in the mass spectrum, because they were completely buried under the signals from an unknown polymer (Fig. 3a). However, electrically biasing the quadrupole mass analyzer essentially removed all the interfering polymer ions and revealed the protein signals (Fig. 3b).

Since peptides and small proteins usually have less kinetic energies than large proteins, the former can also be selectively removed to enhance the spectra of the latter, whose ion signals sometimes are much weaker than those of the former. For example, the signals of the degradation fragments (13.4, 23.6 and 47.3 kDa) (Fig. 4a) of anti-(α1-acid glycoprotein) have been removed from the spectrum of this 150 kDa IgG class antibody (Fig. 4b).
The above studies demonstrate that (1) concomitant non-protein ions have less kinetic energy than protein ions, and (2) this kinetic energy difference can be used to clean up protein mass spectra, and (3) the method is readily applicable on commercial instruments.
Thermally Induced Dissociation of Large Ions from Electrospray Ionization. Alan L. Rockwood, Mark Busman, Harold R. Udseth and Richard D. Smith. Pacific Northwest Laboratories, Richland, WA 99352

Introduction:
Using a heated metal capillary inlet for the sampling of the plume from an electrospray source, we have been able to achieve controlled desolvation and fragmentation by the careful control of capillary heating. The fragmentation processes yield fragment ions similar to those observed by dissociation in the interface and by low energy CID in triple quadrupole experiments.

Experimental:
The thermally induced dissociation (TID) studies were performed on a TAGA 6000E triple quadrupole mass spectrometer. The inlet region has been modified to afford differential pumping between the capillary inlet and the skimmer entrance into the "Q0" region of the mass spectrometer. The electrospray apparatus used a coaxial arrangement with analyte flowing through the center of the apparatus, while being surrounded by a sheath flow of methanol. A small flow of air around the liquid helps stabilize the electrospray current. The heated capillary was constructed of stainless steel (10 cm long, 0.0625" o.d., 0.020" i.d.). The capillary is attached to the TAGA front end by a specially constructed flange. The capillary is resistively heated (50A DC power supply, HP Model 6259B). A schematic of the interface is shown in Figure 1.

Results:
Figures 2a and 2b show spectra for melittin. Figure 2a corresponds to moderate heating (25 W, 200 C, "warm"). Figure 2b corresponds to strong heating (50 W, 350 C, "hot"). The "warm" spectrum was acquired with no potentials applied between the capillary and the skimmer for the stimulation of collisional desolvation in the interface. Effective desolvation has been done by application of heat. The "hot" spectrum was, again, acquired with no capillary-skimmer bias. The extensive fragmentation was achieved by the application of heat through the capillary. The progressive attenuation of the parent ion peaks for a variety of molecules, along with the corresponding appearance of fragment ions, has been observed upon application of stronger heating. At the highest degree of heating the spectra show the collapse of the current into an atmospheric pressure  
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<tbody>
<tr>
<td>vacuum</td>
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</table>

Figure 1
Figure 2a: melittin: warm capillary (−200°C)

Figure 2b: melittin: hot capillary (−350°C)

unresolved envelope of decomposition products. The additive effect of thermal and collisional activation of ions in the interface region has also been observed. Similar degrees of fragmentation can be obtained by various combinations of thermal and collisional heating.

Conclusions:
Ions can be thermally dissociated. The amount of activation energy deposited is probably independent of the ion’s charge state (as opposed to methods for collisional heating in the interface). The role of coulombic forces in the dissociation of multiply charged ions becomes evident, since ions are activated to similar “temperatures” regardless of charge state. The TID technique provides a convenient method for producing fragment ions for further dissociation in a tandem instrument, and provides a basis for kinetic studies.

This work was supported by the U.S. Department of Energy under Contract DE-AC06-76RLO 1830.

*Pacific Northwest Laboratories is operated by Battelle Memorial Institute.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Protein Structural Effects on Electrospray Ionization Mass Spectra and Tandem Mass Spectra

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Pacific Northwest Laboratory
P.O. Box 999, Richland, WA 99352

Mass spectrometry (MS) and tandem mass spectrometry (MS/MS) are important tools for primary structure determination. Electrospray ionization (ESI) is an efficient method for producing multiply charged ions of large peptides and proteins, especially important for instruments of limited m/z. Tandem mass spectrometry can be applied to multiply charged ions to obtain further structural information. Preliminary evidence indicates that fragmentation processes occur primarily from regions remote to the likely charge sites. Comparison of the MS/MS spectra of different charge states of a protein can provide further insight into the fragmentation processes of multiply charged ions.

In addition to the charge state, higher order (secondary, tertiary, quarternary) structure of the protein has an effect on the ESI-MS and (possibly) MS/MS spectra. Changes in the ESI mass spectra (e.g., a bimodal charge distribution) have been observed by changing the solution pH, the organic solvent composition, or by the addition of other denaturants for polypeptides such as ubiquitin (M_r 8564), lysozyme (M_r 14306), and myoglobin (M_r 16950). Such changes in the proteins' solution environment are known to induce conformational changes, i.e., effect three-dimensional structure. Cleavage of disulfide bonds with reducing agents such as 1,3-dithiothreitol also changes the three-dimensional structure of a protein. More charges are typically observed in the resulting ESI mass spectrum and differences are observed in the collisionally activated dissociation (CAD) mass spectrum. Reduction of disulfide bonds arguably allows a compact, globular form to stretch to a more extended conformation, resulting in a shift in the charge state distribution to higher charge. Regions formerly enclosed by Cys-Cys bonds can be probed by CAD. Our results clearly suggest that conformation state can be monitored by ESI-MS, and also suggests the role of tandem MS for further probing of higher order structure.

Analysis of polypeptides and proteins by ESI-MS has generally been performed in the positive ionization mode. The more basic amino acid residues (arginine, lysine, histidine, and the amino-terminus) have dissociation constants such that proteins in acidic solutions (pH < 3) will generally be more positively (protonated) charged. We have observed an approximate correlation between the maximum positive charge observed in polypeptide ESI mass spectra and the number of basic residues, although we (and others) have also observed a poorer correlation for disulfide-rich proteins (due to cysteine-cysteine linkages). Not surprising, most polypeptides in unbuffered aqueous solutions do not afford an equivalent extent of multiple charging in the negative ionization mode. Acidic residues such as glutamic acid and aspartic acid in proteins have pK_a's typically around 5 or less.

Multiply charged anionic molecular ions can be produced in basic pH aqueous solutions by ESI. Horse heart myoglobin (M_r 16950.7) in 1% NH_4OH (pH 10-10.5) yields an electrospray ionization mass spectrum with a bell-shaped multiple charge distribution to approximately the (M-19H)^19- charge state. Molecular weight determination from the same method of calculation routinely used for positive ions but modified for proton loss rather than proton addition, yielded an M_r value of 16949.7 ± 1.2. Also observed in the mass spectrum are ions corresponding to the heme cofactor attached to the polypeptide chain. Even though the heme group is non-covalently bound to the peptide in solution, under certain conditions, species for the heme-peptide are observed in ESI mass spectra. Horse myoglobin has 13 Glu and 7 Asp residues, closely corresponding to the maximum negative charge observed.
Figure 1 is a negative ion ESI mass spectrum for an aspartic proteinase found in gastric juice, pepsin (porcine, M_r 33.6 kDa). Pepsin does not afford a positive ion ESI mass spectrum with our limited m/z range quadrupole instrumentation, while limited success for pepsin has been reported for other ionization/desorption methods such as CI-252 plasma desorption and matrix-assisted laser desorption. Porcine pepsin has only 2 Arg, 1 Lys, and 1 His residues, not nearly enough basic residues for production of multiply (positively) charged ions below m/z 2000. However, 29 Asp and 13 Glu acidic amino acid residues allows multiple charging in the negative ionization mode to the (M-42H)^{42-} charge state with the protein in an aqueous 1% NH₄OH solution. A variety of peptides and proteins up to M_r 66 kDa (serum albumin) and, in addition, small polysaccharides, have been successfully analyzed by negative ion ESI-MS. We are currently exploring the utility of MS/MS of multiply charged negative ions for structural analysis.

Pacific Northwest Laboratory is operated for the U.S. Department of Energy by Battelle Memorial Institute under Contract DE-AC05-76RLO 1830.
Electrospray ionization (ESI) has been employed as an ion source for gas phase ion-ion reaction studies carried out in the high pressure inlet to a quadrupole mass spectrometer. Previously, ion-ion reaction studies were limited to small species relevant to plasma, flame, and atmospheric chemistry. Because ESI efficiently transfers ions from solutions of non-volatile species into the gas phase, the size range of ions available for reaction studies is dramatically extended. In these investigations, multiple ionization sources are used in an arrangement merging unipolar streams of positive and negative ions at near atmospheric pressure. One or both of the reactants are highly charged macroions which undergo partial neutralization or charge inversion to yield charged products.

ESI-generated protein ions, \((M+nH)^{n+}\), react with negatively charges species generated by a discharge, resulting in a shift of the cation charge state distribution to lower charge (higher \(m/z\)), due to proton transfer. The ion intensity maximum in the ESI charge state distribution of horse heart myoglobin (\(M_r 16951\)) shifted from \((M+19H)^{19+}\) to \((M+14H)^{14+}\) after reaction. For horse heart cytochrome c (\(M_r 12,360\)) (Figure 1) the maximum shifted from the 15+ to the 13+ charge state. Some loss in overall ion current is observed after reaction; at least part of that loss arises from our mass spectrometer's lower sensitivity at higher \(m/z\). A relatively greater loss was observed in the myoglobin partial neutralization (total ion current from all charge states reduced by about a factor of 20) than in that for cytochrome c (reduced by a factor of 3). In these ion-ion reactions, attenuation of the high charge states is commonly observed; enhancement of the lower charge states is also observed. In some systems, e.g., myoglobin, the observation of low charge state enhancement is dependent on the capillary-skimmer bias, in part because the ion-ion reaction products show increased solvation and adduct formation. Studies employing dual ESI sources to react \((M+nH)^{n+}\) cations with \((M'-mH)^{m-}\) anions have displayed qualitatively similar behavior, also shifting the charge distributions to lower charge states. The combination of negative ESI source and positive discharge has been investigated, as well. Negative ESI of oligodeoxyadenylic acid, \(p(dA)_9\), yields primarily dianions, with lesser amounts of \((M-H)^-\) and \((M-3H)^-\). Operation of the positive discharge reduces the intensities of the \((M-3H)^-\), \((M-2H)^-\), and \((M-3H+Na)^2-\) ions, while increasing the intensity of \((M-H)^-\).

Collisionally activated dissociation (CAD) of electrospray generated ions occurs efficiently in our instrument at elevated capillary-skimmer biases. However, under the same conditions but with the discharge source operating fragmentation is reduced. This reduction has been observed for \((M+nH)^{n+}\) ions in combination with negative discharge operation and for \((M'-mH)^{m-}\) ions with positive discharge operation, consistent with a partial neutralization reaction occurring in the inlet. CAD occurs in the capillary outlet-skimmer region of the instrument preferentially for higher charge states because of their higher translational energies. If the partial neutralization reaction occurs in the inlet, the number of highly charged ions most likely to fragment is therefore reduced prior to reaching the outlet-skimmer region, resulting in less fragmentation.

Evidence of novel charge inversion from ion-ion reactions has been obtained in dual ESI source experiments. Protonated adenosine 5'-monophosphate (AMP) and fluorescein molecules \((M+H)^+\) are observed as products in the reaction of highly charged macroions \((M+nH)^{n+}\) with species generated in the negative electrospray ionization of AMP or fluorescein. The \((M+H)^+\) products most likely arise from charge inversion of \((M-H)^-\) anions. Protonated fluorescein was observed as a product of reaction with multiply protonated myoglobin (Figure 2) and cytochrome c, and to a smaller extent with melittin, but not with the less highly charged serine and bradykinin ions, or with positively charged species of lower proton affinity from an air discharge. Production of protonated AMP was also only observed for reactions with highly protonated reactants.
In addition to possible analytical applications, these techniques hold promise for future investigations of proton transfer and reactivity with gas phase macrolons.

Pacific Northwest Laboratory is operated for the U.S. Department of Energy by Battelle Memorial Institute under Contract DE-AC06-76RLO 1830. This research has been supported internally by the Molecular Sciences Research Center and the Environmental and Molecular Sciences Laboratory at Pacific Northwest Laboratory.
Simultaneous Determination of the Acid and Lactone Forms of Three HMG CoA Reductase Inhibitors in Plasma by Ionspray Mass Spectrometry.
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Bristol-Myers Squibb Pharmaceutical Research Institute New Brunswick, New Jersey 08903

Pravastatin, lovastatin and simvastatin are three compounds which inhibit the rate-limiting step of cholesterol synthesis, HMG CoA reductase, and have proven efficacious in the treatment of hypercholesterolemia. Pravastatin is administered as the active acid, while lovastatin and simvastatin are administered as lactones and hydrolyzed in vivo to their active forms. The analyses based on GC/MS methods (2-4), despite their high sensitivity, are usually time-consuming, require derivatization and don't permit analysis of acid and lactone forms simultaneously. The HPLC method (1) has insufficient sensitivity and selectivity. We developed a simple, fast and selective assay for simultaneous determination of the acid and lactone form of each of these compounds in plasma/serum by LC/MS using pneumatically assisted electrospray (ionspray) as the MS technique. Use of HPLC eliminated the need for derivatization and sample vaporization for injection as required for GC based methods. In addition it reduced the possibility of acid-lactone interconversion.

Plasma/serum samples spiked with the internal standards were extracted on C2 solid phase cartridges using acetonitrile as eluent and dry extracts were dissolved in loading mobile phase. Plasma samples were stored frozen at -20°C until analysis. The separations were carried out by reversed-phase gradient chromatography on a microbore column DELTABOND C8, 100x2 mm, 5 μm particle size, in the system acetonitrile-water, containing 5 mM of ammonium acetate. Gradient conditions were slightly different for three pairs of analytes: for lovastatin, acetonitrile increased from 30% to 80% in 5 min; for simvastatin, from the initial 32.5% to 80% in 4.5 min and for pravastatin, from 15% to 83% in 5.5 min. The column was maintained at 60°C, flow rate 0.3 mL/min with split of 1:6. All compounds eluted within six minutes and the total analysis time was about nine minutes. The mass spectrometer was operated in the positive ion mode at a low orifice voltage, 45 V, and ammoniated molecular ions were monitored. The system was linear from 5 ng/mL to 1000 ng/mL. The quantifiable limit was about 2 ng/mL for the lactone and 5 ng/mL for the acid forms of all analyzed compounds. Internal standards used are shown in the table:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Internal Standard</th>
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<td>Compactin</td>
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<tr>
<td>Lovastatin acid</td>
<td>Simvastatin acid</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>Compactin</td>
</tr>
<tr>
<td>Simvastatin acid</td>
<td>Lovastatin acid</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>Compactin</td>
</tr>
<tr>
<td>Pravastatin acid</td>
<td>SQ 31,900*</td>
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</table>

* SQ 31,900 is a tetrahydro-analogue of pravastatin acid.

The method presented here is simple, fast, selective and reliable. It permits the simultaneous determination of three pairs of HMG CoA reductase inhibitors in plasma/serum in a wide working range with good sensitivity.
Full Scan Ionspray Mass Spectra of Lovastatin Acid and Lovastatin Lactone

Mass Chromatograms of Lovastatin Acid and Lovastatin Lactone in Plasma Extracts

The biotransformation of xenobiotics may result in the formation of electrophilic metabolites that react with nucleophilic sites in macromolecules. Electrophilic attack of DNA is believed to be an initiation step in a process that may ultimately result in carcinogenesis or mutagenesis, while the analogous reaction of proteins may result in idiosyncratic reactions and cytotoxicity. Hemoglobin has been found to be a particularly useful protein for monitoring tissue exposure to electrophiles because of its availability and its long biological lifetime, which permits determination of cumulative doses. Studies with intrinsically reactive or metabolically activated adducting agents have shown a proportionality between specific adducts of hemoglobin and of DNA. In contrast to DNA, adducted hemoglobin is not generally repaired over the lifetime of the protein. Thus, the monitoring of hemoglobin adducts appears to represent a convenient and sensitive method for assessing tissue exposure to potentially harmful electrophilic metabolites. The characterization of the molecular structure of these adducts may represent a generally useful approach for studying metabolic pathways of xenobiotics. We report here the evaluation of electrospray mass spectrometry (ESI MS) for the rapid and sensitive detection and molecular weight characterization of human hemoglobin adducts of electrophilic metabolites of xenobiotics, using N-acetyl-p-benzoquinone imine (NAPQI) and p-benzoquinone (BZQ) as model electrophiles. Evidence has previously been presented that NAPQI and BZQ are in vivo metabolites of acetaminophen (APAP), and BZQ is an in vivo metabolite of benzene.

EXPERIMENTAL: Human hemoglobin was reacted in vitro with BZQ or NAPQI in whole blood, erythrocytes suspended in phosphate buffered saline (PBS), or free hemoglobin in PBS. Erythrocytes were washed successively with reduced glutathione (5 mM) in PBS and PBS before being lysed in ice cold water. Globin was isolated by addition of hemoglobin to hydrochloric acid/acetone (1/99, v/v), washed successively with acetone and diethyl ether, and stored frozen at -20°C after air drying. The globin was chromatographed using gradient elution reversed-phase HPLC (Vydac C18, 330 Å, 4.6 mm i.d. x 25 cm) and a water/acetonitrile mobile phase containing 0.1% TFA. Chromatographic fractions were lyophilized and dissolved in methanol/water containing 2% acetic acid at concentrations ranging from 5-100 pmole/μL. ESI MS were recorded on a Hewlett-Packard Model 5988A mass spectrometer equipped with an Analytica of Branford, Inc. ESI source. RESULTS AND DISCUSSION: Comparison of the UV absorbance of unadducted versus adducted globin chains at 214 nm and 303 nm indicated reaction of the α- and δ-chains, but no detectable reaction of the β-chain (e.g., Figure 1). An adduct of a component that eluted prior to the major β-chain (pref) was also observed. Plots of the UV absorbance ratio (303 nm/214 nm) of the globin chains indicated a nonlinear increase in the extent of reaction of globin with increasing concentrations of electrophile. Whole blood, erythrocytes suspended in PBS and free hemoglobin reacted in a similar manner, except for quantitative differences in the extent of adduction. The ESI mass spectra of all of the globin chains had a bimodal distribution of ions under the ESI conditions used, irrespective of whether the chains were adducted by BZQ or NAPQI (e.g., Figure 2). The distribution of ions appeared to be slightly different for the adducted β-chains than for the unadducted β-chain. The molecular weights of the globin chains were determined with a precision of ±0.04% or better, and the average molecular weights of the α-, β- and δ-chains were in agreement with the known sequence of these chains. The average molecular weights of the BZQ and NAPQI adducted β-chain were 108 Da and 149 Da higher, respectively, than the unadducted chain. These results are consistent with the formation of hydroquinone and 4-hydroxyacetanilide adducts following reaction with BZQ and NAPQI, respectively. The pref component had an
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average molecular weight that was 305 Da higher than that of the β-chain, suggesting that this was a glutathionyl adduct of the β-chain (theoretical mass 306 Da higher than unadducted chain). The average molecular weight of the BZQ adduct of the preβ component was 107 Da higher than the preβ component, which is likely a 2,5-dicysteinyl hydroquinone adduct based on previous studies of the reaction of BZQ with glutathione.9 The results of these studies clearly demonstrate the utility of ESI MS for the detection and molecular weight characterization of hemoglobin adducts of electrophilic metabolites of xenobiotics.


Figure 1. Chromatograms of Globin Isolated from Control and BZQ Adducted (3.15 mM) Erythrocytes.

Figure 2. Electrospray Ionization Mass Spectrum of BZQ Adducted Hemoglobin β-chain. Average Calculated Molecular Weight 15976 ± 6 Da (±0.04%).
Electrospray ionization-mass spectrometry (ESI-MS) is rapidly becoming a practical biochemical tool for peptide and protein analysis. Production of multiply charged molecular ions allows conventional mass spectrometers of limited m/z range to analyze proteins with relative molecular mass (M_r) greater than 100 kDa with precisions of better than 0.005%. The utility of ESI is further enhanced by the increased efficiency of multiply charged ions for collisionally activated dissociation (CAD), often yielding multiply charged product ions that can be correlated to the amino acid sequence. Dissociation of the multiply charged ions can be induced either in the atmospheric pressure/vacuum interface (nozzle-skimmer region) or in the collision quadrupole cell of the triple quadrupole mass spectrometer. Human hemoglobin (Hb, ~62 kDa) consists of 2 a chains (15.1 kDa, 141 residues) and 2 non-a chains associated with a prosthetic heme group. Hemoglobin variants, of which over 400 are known, are characterized by amino acid substitutions in the globin polypeptide chain. We have investigated ESI-CAD-MS of eight intact 3-globin variant polypeptides as a tool for potential rapid analysis of these amino acid substitutions. The approximate location of the modification can be deduced from comparison of the CAD mass spectra and the observance of mass shifts of the fragment ions containing the substituted amino acid. Also, the effects of these amino acid substitutions on the CAD mass spectra was investigated. The effect of residues such as proline on collisional dissociation data for multiply charged parent ions has been previously noted by our laboratory. The fragmentations observed for these Hb 3-globin variants are dominated by cleavage on the NH2-terminus side of proline residues present in the polypeptide chain. One major site of fragmentation of the Hb 3 chain variants is the NH2-terminus of the proline at residue 51. Complementary ions are observed from this fragmentation process as highly charged (+8 to +13) b_{50} and y_{66} fragment ions. These complementary fragment ions are not observed for the Willamette variant as it has a substitution at residue 51 of an arginine for the proline, and therefore does not fragment at that site. Other variants studied with amino acid substitutions covering various molecular regions showed no differences in the CAD mass spectra. The majority of the dissociation products observed are assigned as fragment ions of relatively low charge state (+1 to +4) based on comparison of the MS/MS and interface dissociation spectra of the variants. Extensive fragmentations at the COOH-terminus of the polypeptide chain are observed as singly, doubly, and triply charged "y_n" fragment ions. A series of doubly charged "b_n" fragment ions from the NH2-terminus in addition to a singly charged b_4 fragment ion from cleavage of the -CO-N- backbone bonds are present in the dissociation mass spectra. The majority of the other fragment ions observed are centered around proline residues.
Human Hemoglobin β^A Chain (M_r 15,867.2)

MS/MS

Hemoglobin β^A Chain

(M+18H)^18^+
Electrospray Ionization Mass Spectrometry of Recombinant Proteins.

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The recent application of the Electrospray ionization (ESI) technique to the field of proteins has allowed many laboratories to determine the molecular weights (MW) of proteins on instruments of limited mass range \((1,2)\). Recently we have acquired an electrospray ion source from the Vestec Corp. for our Vestec 201A LC/MS instrument and applied the ESI technique to the determination of the MW of bovine and porcine recombinant somatotropins (rbST and rpST) and some of their analogs at position 99 \((3)\). In this work we determined the centroids of the various clusters manually and calculated the number of charges, the MWs and the average MWs off-line. This method is long, tedious, prone to human errors and consumes a lot of the sample under investigation.

Since then we have computerized the data processing step so that the data acquired on the Teknivent Vector 1 system are transferred to the Harris\textsuperscript{7} dual CPU computer and processed. Two methods of calculating the average MWs have been developed in our labs: one using the centroid determination \((4)\) and the other one using a deconvolution algorithm as described in the literature \((5)\). In general the accuracy of the data is similar to the manual method, although the deconvolution method seems to give more accurate results in most cases. Several facilities have been incorporated in these programs: smoothing of noisy scans, manual picking of the clusters of a series if necessary, deletion of certain peaks that are due to noise, subtraction of the peaks that have been interpreted, etc. In the deconvolution method the abundances belonging to a given cluster series are summed and displayed on the mass scale of the protein. Thus the peak areas reflect the abundances of the proteins present as well as of impurities and adducts formed. The average MWs of the peaks, even small ones, can be easily determined by positioning the cursor at the center of a given peak displayed on a CRT screen.

We have expanded this research to other proteins of interest to our research community. Thus we have analyzed several homologs of recombinant bovine somatotropin (rbST) in which an additional amino acid has been inserted and in which one or more amino acids have been changed. Insertion of a serine between the threonine at position 98 and the aspartic acid at position 99 of rbST-99D yielded a partial sequence of -98Thr-99Ser-100Asp-101Ser-. The theoretical average MW of this protein is 21,900. The found values were 21,901.3 and 21,900.5. When the aspartic acid in the previous sequence was replaced by serine, the following partial sequence -98Thr-99Ser-100Ser-101Ser- was the result. The theoretical average MW for this protein is 21,872.0 and the found values are 21,874.3 and 21,871.5.

The mixtures of natural growth hormones from the pituitary glands of cows pigs and humans have also been analyzed by this technique. The ESI mass spectrum of the pituitary bovine

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somatotropin (pbST) is shown in Fig.1. From this it is apparent that two major proteins are present in this mixture. The average MWs of these two proteins were determined as 21,812.5 and 21,425.0. Thus the higher MW protein corresponds to bovine somatotropin (theory 21,812.0) while the lower MW corresponds to bovine somatotropin which has lost 4 amino acids from the amino terminus (bST-AFPA). A similar analysis has allowed us to assign the other peaks to sulfate/phosphate adducts or losses of amino acids from the amino terminus or a combination of both.

Fig. 1. Electrospray Ionization Mass Spectrum of Pituitary Bovine Somatotropin 100ng/ul in MeOH/H2O/HOAc (47.5/47.5/5.0), Infusion at 1ul/min.

DEGLYCOSYLATION OF INTACT PROTEINS WITH SUBSEQUENT ANALYSIS BY ELECTROSPRAY IONIZATION MASS SPECTROMETRY.


Mass spectral analysis of glycoproteins can identify or confirm the structures of both polypeptide and oligosaccharide portions of these important molecules. However, heterogeneity present in the carbohydrates may decrease the sensitivity of analysis of intact glycoproteins by electrospray ionization mass spectrometry or may prevent detection altogether. Previous work on identification of proteins following deglycosylation has been done at the peptide level. Removal of the oligosaccharides therefore facilitates mass spectrometric detection of glycoproteins. Subsequent measurement of the polypeptide mass provides answers questions to regarding the fidelity of expression of recombinant proteins, and the presence of backbone clips or processing. In this work, chemical and enzymatic methods for removing N-linked and O-linked carbohydrates were compared. Ribonuclease B was used as the primary standard for deglycosylation with hydrofluoric acid (HF), trifluoromethanesulfonic acid (TFMSA), endo H, and PNGase F.

Few glycoproteins yield interpretable mass spectra without deglycosylation. One exception is ribonuclease B (Mr 14,900-15,550) where the two major and three minor glycoforms give distinct masses for each (Figure 1). Deglycosylation of this protein was accomplished by enzymatic methods (Endo H and PNGase F) and acid cleavages (HF and TFMSA). The product of cleavages by endo H, HF and TFMSA retain one N-acetylglucosamine residue attached to the asparagine. Starting material in these cases containing both glycosylated (ribonuclease B) and non-glycosylated (ribonuclease A) forms of the protein. The products observed from chemical deglycosylation gave a broad cluster of peaks in the region of protein with a single GlcNAc attached. PNGase F digestion was successful at the 20 picomole level and endo H was successful at the 10 picomole level (Figure 2). The products were introduced into the mass spectrometer using HPLC for best signal to noise and peak width.

The mass spectrum of the intact bovine fetuin, which contains three N-linked and four O-linked oligosaccharides, was not interpretable without prior knowledge. However, sequential removal of the sialic acid residues, N-linked and most O-linked oligosaccharides using neuraminidase, PNGase F and O-glycanase produced a mass spectrum where the protein mass could be determined.

Methods for isolating and desalting the protein cores varied depending on the hydrophobicity of the product. Reversed-phase HPLC was preferred if solubility allowed, and best results were obtained by capillary LC/MS. Alternatively, many deglycosylated proteins are very hydrophobic (eg. DNase), and poorly soluble in aqueous/acetonitrile solutions. Salts and surfactants were removed by precipitation of DNase and it was dissolved in 100% DMSO for electrospray ionization mass spectrometry (Figure 3). The protein remained intact during this procedure as shown by the intact mass.

In summary, removal of the N-linked and O-linked oligosaccharides allowed masses of the amino acid portion of the glycoproteins to be determined with good accuracy. Limitations to the method include the lack of a universal enzyme for the removal of O-linked oligosaccharides, and poor solubility of many deglycosylated proteins.

Figure 1. Ribonuclease B contains five molecular species at Mr 14,899, 15.061, 15.224, 15.366, and 15.548 kDa. Each glycoform differs from the next homolog by a mannose residue (+162 Da).

Figure 2. Digestion of ribonuclease B with endo H produced protein masses corresponding to a single GlcNAc residue (B, 13.885 kDa). PNGase F digestion gave similar results. (data not shown) and Deglycosylations were carried out using 10 picomoles (endo H) or 20 picomoles (PNGase F) of starting glycoprotein, and the solutions were desalted and introduced into the mass spectrometer by capillary LC/MS.

Figure 3. The electrospray ionization mass spectrum of recombinant DNase following digestion with PNGase F. The precipitated protein was ionized from 100% DMSO. The peak top is shifted to a mass slightly higher than theoretical (as shown by the lines) due to residual salts.
Electrospray Analysis Of Ribonuclease B: Observation Of Glycoforms

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Bovine pancreatic ribonuclease B (RNase B) is a 124 amino acid glycoprotein of 13,700 protein mass with a single N-linked glycosylation site containing a series of structurally defined high-mannose oligosaccharides. The commercial availability of RNase B as well as the structural simplicity of its oligosaccharides makes the glycoprotein a convenient and common choice for the development of carbohydrate analytical techniques. We have attempted to validate the utility of electrospray MS for the characterization of oligosaccharide structures on an intact glycoprotein using RNase B as a model.

Electrospray MS analyses were performed by continuous infusion of 100 ng/μL solutions of RNase in 5% acetic acid in 20:80 methanol:water into a Vestec Model E200 Electrospray interface. Each spectrum is the average of three 10 second scans, corresponding to the consumption of 17.5 pmol during the analysis. Electrospray MS analysis of "pure" RNase B (Fig. 1), obtained from Sigma, indicated the presence of a complex mixture dominated by multiply-charged ions (labeled A) from the non-glycosylated form RNase A. Also observed were multiply-charged ions (labeled B) which were assigned to RNase B. The "pure" RNase B sample was then purified by Sigma to generate a second RNase B, "ultrapure" B. ESPMS analysis of "ultrapure" RNase B (Fig. 2) demonstrated the absence of RNase A and the presence of multiply-charged ions indicative of a mixture of nine components. The difference in mass between the two major components, 1 and 4, was 162 amu, which corresponds to one mannose unit. The masses observed for 1 and 4 were consistent with oligosaccharide components Man₅GlcNAc₂ and Man₅GlcNAc₂, respectively, on RNase. The mass differences between components 4 and 7 (160 amu), 7 and 8 (166 amu), and 8 and 9 (160 amu) suggested the presence of additional mannose units. Components 2 and 5 were identified as acetate adducts to species 1 and 4, respectively.

To further characterize the glycoforms of RNase B, "pure" and "ultrapure" RNase B were analyzed by reverse phase HPLC, CZE, and SDS-PAGE. HPLC and SDS-PAGE analyses indicated the presence of 2 components (RNase A and RNase B) in the "pure" sample and one component (RNase B) in the "ultrapure" sample. In the CZE analyses, 3 components (RNase A, Man₅RNase B and Man₅RNase B) were observed in the "pure" sample and 2 components (Man₅RNase B and Man₅RNase B) in the "ultrapure" sample.

To validate the ESPMS results, "ultrapure" RNase B was subjected to classical sugar analysis. N-linked oligosaccharides were released from RNase B by treatment with anhydrous hydrazine. After purification by paper chromatography, the oligosaccharides were labeled by reduction with ²H-borohydride and subjected to gel permeation chromatography on Biogel P4 (Fig. 3). The major oligosaccharides eluted at 9.0 and 9.9 glucose units, characteristic of Man₅GlcNAc₂ and Man₅GlcNAc₂ species, respectively. These sugars coeluted with standard oligosaccharides, and both yielded the Man₅GlcNAc₂ species (5.5 glucose units) upon treatment with Jack Bean alpha-mannosidase, indicating that they both contain the beta-linked mannose core, Man(bet1-4)GlcNAc(bet1-4)GlcNAc(bet1-4)GlcNAc-OH and 4 or 5 outer alpha-linked mannose residues.
FIG. 1  ESPMS ANALYSIS OF "PURE" RNase B

FIG. 2  ESPMS ANALYSIS OF "ULTRAPURE" RNase B

FIG. 3  P4 CHROMATOGRAPHY
SELECTIVE DETECTION OF GLYCOPEPTIDES IN PROTEIN DIGESTS BY ELECTROSPRAY LC MS/MS

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Locating glycosylated peptides in an HPLC map of a protein digest is a frequently encountered task in protein analysis. As mass spectrometry is called on to confirm structure and map post-translational modifications of increasingly larger proteins, techniques that rely on changes in HPLC pattern after removal of the carbohydrates to identify potential glycopeptides become less reliable. Furthermore, the characteristic multiple-peak signature of a glycopeptide arising from heterogeneity in the carbohydrate may be obscured by coeluting peptides that are often present in significantly higher concentration than any of the individual glycoforms. Clearly, more selective detection methods are required. Toward this end we have investigated the utility of constant neutral-loss and parent-ion scans on a triple quadrupole mass analyzer during electrospray MS and LC-MS.

MODEL STUDIES ON OLIGOSACCHARIDES AND ISOLATED GLYCOPEPTIDES: A pure oligosaccharide, Man$_4$GlcNac, as well as glycopeptides derived glycopeptides containing high-mannose or complex oligosaccharides were used as model compounds to study the use of different scan modes for glycopeptide-specific detection and to optimize the ESMS system parameters for sensitivity.

NEUTRAL-LOSS SCAN MODE: The neutral-loss scan was less informative than the parent-scan mode. Since the mass of a neutral loss is affected by the charge state of the parent ion, this type of MS/MS experiment is, in general, less useful in ESMs.

PARENT-SCAN MODE: These experiments provided useful information. The carbohydrate-related fragments are lost preferentially from the protonated ion species. Therefore the parent-scan data has the appearance of the normal ESMs data yielding predominantly MW information, but it is selectively obtained for glycopeptides. Most importantly, the information is obtained regardless of charge state.

The following oxonium fragment ions were evaluated in parent scan mode: m/z 147 (Fuc), m/z 163 (Hex), m/z 204 (HexNac), m/z 232 (NeuAc) and m/z 366 (HexHexNac). The m/z 163 and m/z 204 gave useful data with the pure oligosaccharide and the oligomannose glycopeptide, and m/z 204 and m/z 366 gave useful data for the complex glycopeptide. Generation of the m/z 204 HexNac fragment appears to be general for any carbohydrate or glycopeptide containing HexNac in the inner core. Like peptide immonium-ions, this fragment arises from that population of molecules that is extensively fragmented upon collisional activation. At present we are not sure if the 204 is arising selectively from one of the two inner core HexNac’s. Because the m/z 204 is generic to all of the common glycopeptide structural types it was chosen for the LC-MS/MS experiments.

LC/MS/MS OF PROTEIN DIGESTS FOR LOCATING GLYCOPEPTIDES: Parent scanning of m/z 204 (HO-HexNac$^+$) has been used to locate glycopeptides in the LC-MS/MS data of three glycoproteins. (1) CD4, the receptor for the HIV-virus: two sites of glycosylation; (2) gp120, the 85 kDa surface glycoprotein of the HIV-virus: 24 potential sites of glycosylation; and (3) soluble complement receptor, a 250 kDa glycoprotein containing 25 potential sites of glycosylation (Fig. 1). The mass spectra from scans at which the TIC of the parent ion scan shows a response were examined to see if the response was due to glycopeptide. In most cases the spectra (for example, see Fig. 2) contained series of molecular weight-related signals separated by the mass increments of hexose, sialic acid, deoxyhexose, etc. Multiple signals for the same glycopeptide are observed because the carbohydrate moieties are often heterogeneous, but this heterogeneity does not affect the retention behavior on RP-HPLC to a great extent. Parent ion scanning for m/z 204 occasionally detected peptides that could fragment to yield an abundant m/z 204 (typically as a y$_n$ or b$_n$ fragment).

SUMMARY: LC/MS/MS VS. LC/MS FOR LOCATING GLYCOPEPTIDES IN COMPLEX MIXTURES

* LC/MS/MS in parent scan mode enables glycopeptides to be located rapidly in complex digests.
* Interference from co-eluting peptides which can obscure the presence of the glycopeptides is eliminated.
* The same carbohydrate compositional information obtainable from the normal MS scans is available from the parent ion scan data.
* Parents of m/z 204 (HexNac) in LC/MS/MS mode should permit equally facile identification of O-linked as well as N-linked carbohydrates.
* Other parent scan modes (such as parents of NeuAc and Hexose) are useful for distinguishing complex from oligomannose carbohydrates.

In contrast to peak pair searching and "diagonal" analysis of LC/MS data, parent scanning enables glycopeptides lacking heterogeneity to be identified.
Figure 1. (top) Total ion current trace for the LC-ESMS analysis of a tryptic digest of soluble complement receptor. (bottom) Parent-ion scan profile of m/z 204 acquired during an LC-ESMS/MS analysis of the above sample. Separation was carried out on a 1mm C-18 column with a TFA/CH$_3$CN/H$_2$O gradient at a flow rate of 40 µl/min.

\[ (M+3H)^{3+} \]

\[ M_r = 2978 \]

Figure 2. Electrospray mass spectrum of a glycopeptide identified in the LC-MS/MS analysis of a tryptic digest of sCR-1 using parent ion scanning of m/z 204. Spectrum is the sum of scans 273 to 280 of the data set shown in Figure 1, bottom.
CHEMICAL MODIFICATION OF GLYCOPEPTIDES AND PEPTIDES FOR ENHANCING MULTIPLE CHARGING IN ESI-MS.

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Molecular weight determination of high mass peptides by electrospray ionization is one of the most important recent developments in mass spectrometry. It has been shown that the main sites of protonation are the amino group on arginine, lysine, ornithine and histidine residues. For peptides or small proteins with a sufficient number of basic residues, the observed multiple charge states can be used for direct analysis. For tryptic glycopeptides or peptides lacking basic residues their mass/charge ratio may fall outside the mass range of the instrument and their detectability is severely limited.

This presentation describes two procedures for enhancing multiple charging of these peptides by quantitative i) amidation of acidic residues to afford amino group bearing sites, ii) vinylpyridine etheration of cysteine residue. The advantages of increasing the number of charge states on peptides and glycopeptides are threefold:

i) brings the mass/charge ratio within the mass range of the triple quadrupole,
ii) increases the ionization efficiency,
iii) improves the detection of glycoforms in a complex mixture.

These advantages can be best illustrated if we consider the three tryptic glycopeptides derived from bovine fetuin, BF T55-85 (peptide base mass 3402.79), BF T126-141 (peptide base mass 1755.08) and BF T142-169 (peptide base mass 3017.39). They are all glycosylated with various glycoforms of complex-type structures with base mass between 892 to 3300. Therefore in their native form their mass/charge ratio is above the mass range of the triple quadrupole and cannot be effectively analysed by ESI.

After amidation or etheration, these glycopeptides will carry more residues for protonation to bring their mass/charge ratio down to the triple quadrupole mass range. Another added advantage of this procedure is that multiple charging improves the detection of glycoforms in a complex mixtures. This is illustrated with the synthetic ESI spectra of native and amidated BF T126-141. The native glycopeptide (maximum of 3 charge states) after deconvolution gives a lot of erroneous signals while the amidated glycopeptide (maximum of 6 charge states) provides a much better analysis of the spectrum.

Cys-Cys residues are reduced in the normal manner (TRIS buffer, guanidine HCl and dithiothreitol) follow by treatment with 4-vinylpyridine (VP) to afford the thio-ether. The carboxyl groups are quantitatively amidated with N,N-dimethylethlyenediamine (DMED) or with the more reactive 3,3'-diamino-N-methylidipropylamine (DAMDPA), which convert
carboxyl groups to amino group bearing sites. Small peptides are directly amidated as their methyl ester in hexafluoroisopropanol, while larger protein are amidated via a carbodiimide reaction in hexafluoroacetone. The DMED reaction is stoichiometric, while only one of the free amine in DAMDPA is involved in the condensation reaction. Both VP and DMED derivatives carry one charge site, while DAMDPA derivatives carry two possible sites for protonation.

The FAB spectra of the amidated peptides showed very prominent doubly charge molecular ions (sometime the base peak), while the ESI spectra of the derivatized peptides showed more multiple charging than the native material. We have applied these procedures to a variety of peptides and proteins, and find them very straightforward and can be easily incorporated as a standard step prior to ESI analysis.
ELECTROCHEMISTRY AND TANDEM MASS SPECTROMETRY: A DYNAMIC COMBINATION FOR STUDYING BIOLOGICAL REDOX REACTIONS

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The development of methods to provide information concerning the activity, mechanism of action, and metabolism of various drug candidates is an important aspect of the pharmaceutical drug discovery process. Therefore, techniques which can give insights into the mechanism and structural factors which might influence metabolite formation are significant tools. Electrochemical methods have been used successfully to investigate the redox chemistry of biologically significant molecules as well as synthetic compounds with biological activity (1-4). This is based on the fact that both electrochemical and biological redox (oxidation or reduction) reactions involve heterogeneous electron-transfer processes. As a result, electrochemistry in combination with mass spectrometry (EC/MS) is well suited to provide insight into redox and related chemical reactivity of compounds of biological interest.

In these studies, an electrode is poised at a suitable potential and is used to simulate the redox enzyme. The products of the electrochemical reaction are analyzed by mass spectrometry (Figure 1). Although the unique selectivity associated with enzymes cannot as yet be precisely duplicated using this method, in analogy to the active site of an enzyme, the substrate molecule must be properly oriented before an electron transfer reaction can occur at the electrode surface. Buspirone, an anxiolytic drug, was chosen as a model compound due to the extensive data available about its in-vivo routes of metabolism (5).

Figure 2 illustrates the mass chromatograms of the [M + H]^+ ions of buspirone and four oxidation products observed by mass spectrometry using a thermospray interface. Daughter MS/MS spectra were acquired for each of these products and the spectra revealed possible structures for these compounds. Figure 3 illustrates the daughter MS/MS spectrum of an electrochemically generated oxidation product of buspirone. Based on the product profile, two separate oxidation pathways were proposed to account for products identified by MS/MS. Figure 4 shows a comparison of the electrochemically generated products with those metabolites formed in-vivo. These results indicate that EC/MS/MS can be used to provide information concerning metabolism and degradation processes of drug candidates. The formation of these species via electrochemical oxidation of buspirone and detection by mass spectrometry is significant since the same metabolites are formed in-vivo.

REFERENCES

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BLOCK DIAGRAM OF ON-LINE EC/MS/MS SYSTEM

Figure 1

MASS CHROMATOGRAMS OF THE OXIDATION PRODUCTS OF SUSPROME OBTAINED BY HIGHSPIRAY

Figure 2

CORRELATION OF EC GENERATED SPECIES WITH METABOLITES FORMED IN VIVO

Figure 3

Figure 4
Microdialysis sampling combined on-line with electrospray mass spectrometry

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Current protocols for rapid structure elucidation of trace compounds in complex mixtures, which are based on tandem mass spectrometry (MS/MS) and LC/MS/MS, are currently in use in our laboratory. For example, drug metabolites in physiological fluids are currently screened using the parent and neutral loss MS/MS modes, followed by obtaining detailed structural information by daughter MS/MS and LC/MS/MS. In this manner, endogenous and xenobiotic compounds are commonly identified by mass spectrometry at concentrations below 1 microgram/mL in physiological fluids. As part of an ongoing improvement of methods, studies were performed to enhance present trace mixture analysis procedures and instruments to obtain chemical information directly from biological systems.

Microdialysis sampling (1-4) readily interfaces with certain types of mass spectrometers. It provides a method for sampling living animals which causes minimal disruption, permits analysis in real time and may reduce degradation of analytes. We previously described an integrated microdialysis/MS/MS system using the thermospray interface and a series of sampling valves (5). In the present study the use of the electrospray interface has improved the performance of the system.

The system used in these studies is shown in the following figure. A CMA/10 microdialysis probe from Carnegie Medicin was connected directly to the ionspray interface of a Sciex API III tandem quadrupole mass spectrometer. The quantitative capabilities of the system were studied using selected reaction monitoring, in which Q1 was set to selectively pass the (M+H)+ ion of a model compound gepirone and Q3 was set to selectively pass the m/z 122 collision induced fragment ion of gepirone. In this way the MS/MS served to selectively detect gepirone in the presence of a complex matrix without time-consuming chromatographic separation. The capability of the system for MS/MS screening and structure elucidation was performed by dialyzing solutions of gepirone or a plasma sample containing gepirone and metabolites while collecting daughter, parent and neutral loss MS/MS spectra.

The effect of dialysis flowrate on the response of the system was studied by changing the dialysis flowrate from 2 to 20 microliters/min. It was found that the response increased in direct proportion to decreases in the flowrate. This result reflects the fact that with reduced flowrates the dialysis efficiency increases and causes increased concentration of analyte in the dialysate. Electrospray, being a concentration sensitive ionization device, produces greater ion yield as the concentration of the analyte increases. Thus, microdialysis with low flowrates is an ideal match for electrospray ionization. A flowrate of 10 microliters/min was used for the remainder of the studies, for comparability to previous studies. However, these results indicate that at least 10 times greater sensitivity may be achieved at a flowrate of 1 microliter/min.

The detection limit of the system was studied by dialyzing successively lower concentrations of gepirone. A detection limit of about 100 ng/mL was observed.

The limit of identification, the lowest concentration at which a useful MS/MS spectrum could be obtained for structure elucidation, was found by dialysis of successively lower concentrations of gepirone. At a concentration of 200 ng/mL a useful daughter MS/MS spectrum was obtained which contained all of the characteristic daughter ions of gepirone.

The capability of the system for MS/MS structure elucidation was studied by dialyzing a plasma sample in vitro. Using a series of parent and neutral loss MS/MS screens, two
metabolites and gepirone were observed in the plasma. A daughter MS/MS analysis of the metabolite at m/z 376 indicated that this metabolite was hydroxylated on the dione moiety. The pyrimidinyl piperazine metabolite was also readily observed.

An integrated microdialysis/MS/MS system offers advantages over traditional strategies of biological sample analysis. The sample is obtained with a minimum of disruption and degradation. The sample is directly analyzed by the mass spectrometer with immediate production of data each 2 seconds, while in most applications of microdialysis samples are collected for 10-30 minutes and analyzed off-line after sample storage and preparation. Valuable structural information may be obtained from mass spectra using this system. Use of an electrospray interface with the system permits sensitive detection of analytes and low energy ionization for labile molecules. Future applications of this apparatus may include: drug metabolite screening directly from living awake animals, analysis of drugs, metabolites and endogenous compounds in tissues, investigative pharmacokinetics, and monitoring of in-vitro biochemical reactions. The system may be applied to broader experimental strategies involving the simultaneous monitoring of physiological data and chemical events in a living system.

References
Electrospray MS has generated a great deal of interest due to its suitability for the analysis of high molecular weight and thermally-labile compounds. For many compounds, electrospray has been shown to be a gentle ionization technique, providing spectra similar to those from FAB/MS. Electrospray has the additional advantage of generating multiply-charged ions, allowing mass spectrometric analysis of compounds not normally within the mass range of the instrument.

Our interest has been to combine electrospray with small-scale separation techniques designed for the analysis of low levels of analyte. Two such techniques are capillary zone electrophoresis (CZE) and nanoscale capillary LC (75 μ i.d. packed columns) (nCLC), which are characterized by submicroliter per minute flow rates, small injection volumes, and high separation efficiencies. These characteristics make these separation techniques ideal for on-line coupling with mass spectrometry.

Because of the sub-microliter per minute flow rates associated with these techniques, a make-up flow is required for the generation of a stable ESI spray. This supplemental flow is provided coaxially with a modified Vestec ESI probe. Coaxial arrangement of the analytical column and a sheath flow (to provide sufficient flow for generation of the spray) does not compromise chromatographic resolution and permits the independent optimization of sheath flow and analytical mobile phase.

CZE and nCLC have been successfully coupled to ESI/MS and have been applied to a wide variety of mixtures. Separation of synthetic mixtures of peptides, proteins, tryptic digests, and bile acids can be readily achieved using either CZE or nCLC, but the higher loading capacity of the nCLC column proves distinctly advantageous where mass spectrometric sensitivities are limiting. On the other hand, CZE peak widths are narrower, detection limits are lower, and analysis times are shorter.

Separations of closely-related cytochrome C's and myoglobin were achieved by CZE/ESI at the femtomole level, while nCLC separations have been performed on tryptic digests of ACTH and cytochrome C, and bile acids. Mixtures of neuropeptides, angiotensins, and chemotactic peptides could be separated by both techniques. Separations have been achieved on as little as 2 pmoles per component by CZE/ESI/MS and 12 pmoles by nCLC/ESI/MS.
SUBTRACTIVE METABOLIC PROFILING BY CAPILLARY LC-ES-MS IN THE METHODICAL SCREENING FOR SIGNAL AND EFFECOTOR MOLECULES THAT PLAY A ROLE IN FIREBLIGHT DISEASE.

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Fireblight is one of the longest known and most feared plant diseases and is especially destructive to pear and apple. The name is derived from the typical firetorched appearance of the late, necrotic stage of this disease. Toxins of low molecular mass excreted by the causative bacterium, Erwinia amylovora, have long been suspected to contribute to fireblight necrosis because crude extracts from infected tissue or culture filtrates can cause necrosis in cell cultures, shoots, or intact host plants. Previous research by this investigator tentatively identified dihydro-phenylalanine (DHP) as a potential fireblight toxin [Phytochemistry 27, 3417-3422 (1988)]. However, these studies relied on only a few E. amylovora strains and subsequent investigations have revealed that DHP is not constitutively produced by all virulent isolates of E. amylovora. Other, yet unidentified toxins or inhibitors of the hypersensitive reaction (HR) may also contribute to the development of fireblight. Because of its high speed, high sensitivity, high specificity and resolution, low chemical background, ‘soft ionization’, and its feasibility to directly interface with LC [thereby avoiding a drying step that is deleterious for DHP (G. Feistner, 35th ASMS Conference, Denver, 1987)], electrospray (ES) mass spectrometry is extensively employed in our screening for novel bioactive compounds. Here, I report on the identification and partial characterization of siderophore-like compounds from wildtype E. amylovora 1430. Based on their abilities to bind ferric ion, four siderophores have been tentatively identified. Figs. 1a and 1b show the ES spectra of one of them, MW 587, before and after mixing with ferric citrate. The other ligands have molecular masses of 601, 619, and 800. Interestingly, the spectra of the ferric complexes not only show the binding of one ([MW + Fe3+ -2H+]++; e.g., m/z 641, 655, 673), but also of 2 ferric ions ([MW +2Fe3+ -4H+]2++; e.g., m/z 347.5, 354.5). Mass chromatography suggested that the same ligands also can bind aluminum as [MW + Al3+ -2H+]++. The latter was confirmed in experiments in which the siderophore was mixed with AlCl3. Fig. 1c shows the ES spectrum of the ligand with MW 601 after mixing. Moreover, when this mixture was further treated with ferric citrate, the aluminum was not replaced but a mixed ferric/aluminum complex ([601 +Fe3+ +Al3+ - 4H+]2++; m/z 340; Fig. 1d) was formed. MS/MS studies indicate that the ligands are composed of three monomers. To my knowledge, siderophores with such...
characteristics have not yet been described. We may thus have encountered the non-catechol, non-hydroxamate-type siderophores previously identified by Leong and Neilands based on a growth factor assay [Arch. Biochem. Biophys. 218, 351-359 (1982)]. Metabolic profiling by capillary LC-ES-MS was used to demonstrate that the production of the siderophore-like compounds increased under iron-deficiency stress but that their production was minimal and unresponsive to iron-deficiency stress in an avirulent, single site transposon mutant. Fig. 2 gives examples of the mass chromatograms obtained (still subject to improvement) and shows that by using this methodology many more compounds can be identified than would ever be possible with UV detection (bottom panel). The dynamic range is approximately 10^3 which appears to be adequate for the detection of the loss of a biosynthetic capacity or of a coarse regulatory mechanisms thereof. In general, siderophores enable pathogens to grow in the iron-deficient environment of a host tissue and, through binding of free ferric ions, inhibit the production of hydroxyl radicals that are produced by the host plant during the HR in an attempt to kill the pathogen. The observed siderophore-like compounds may thus have a direct bearing on the development of fireblight disease, which is a strong incentive for us to fully elucidate their structures.
Electrospray ionization-mass spectrometry (ES-MS) has gained most of its recent attention because of the ability to produce multiply charged ions from very large biomolecules making them amenable to analysis by most modern mass spectrometers. However, ES-MS is equally well suited for compounds of low or moderate molecular weight that are difficult to volatilize intact by other methods. Moreover, the early work of Fenn and co-workers (1,2) and recent reports by Kedarle and co-workers (3,4) attest to the applicability of ES-MS to the study of the gas-phase chemistry of multiply solvated or coordinated metal ions. The utility of ES-MS for the analysis of metals in solution derives in part from the facility with which the metal ions are solvated by or form complexes with the ES solvent or other reagents added to the solvent. Solvation and complexation can be a hindrance, however, in the analytical application of ES-MS to the analysis of metals in solution, especially solutions of metals in water.

The data presented here demonstrate that many of the problems in the ES-MS analysis of metals can be overcome by complexing the metals with crown ethers and/or extracting the metals from water into an organic phase using crown ethers. This procedure, in addition to transferring the metals to a solvent more amenable to the spraying process, concentrating the metals by reduction in solvent volume (thereby lowering detection limits), and providing the possibility of preferential metal complexation/extraction, usually results in the observation of a single metal-ligand complex of the same charge as the metal in solution when using the appropriate crown ether for the metal(s) of interest.

The data described in this paper were acquired on an ES/ion trap mass spectrometer combination that has been described previously (5). All reagents were obtained from commercial suppliers and used without further purification and all solvents used were HPLC grade. Methanol/water soluble metal salts were used to prepare standard solutions of the metals in water and methanol. Standard solutions of the crown ethers were prepared in toluene.

One of the major problems faced in the analytical application of ES-MS to metal analysis is the formation of metal-solvent adduct ions. These adducts complicate the spectra and spread the analyte signal out over several ionic species thereby raising detection limits. This problem is less severe for singly charged species such as the alkali metals, but for multiply charged species the problem can be quite pronounced. Another problem in the analysis of metals is the loss of charge state information. That is, the charge state of the metal in solution is not always preserved in the gas-phase. As Kedarle and co-workers (3,4) have pointed out, charge reduction of a particular metal ion depends on the relative ionization energies of the metal ions and ES solvents employed. The ES mass spectrum of UO$_2^{2+}$ demonstrates this phenomenon. In this spectrum (not shown), UO$_2^+$ and other singly charged species are observed rather than UO$_2^{2+}$, which is the ionic species in solution.

The first of these problems, and possibly the second, can be largely overcome by complexing the metals in solution with a crown ether such as 12-crown-4 or dibenzo-18-crown-6 (see Figure 1). For example, the ES spectrum of Na$^+$ sprayed from methanol (Figure 2) is complicated by the presence of adduct ions containing both water and methanol. The ES spectrum in Figure 3, obtained from the same solution after addition of the complexing agent dibenzo-18-crown-6, contains only one peak, which corresponds to the Na-crown complex. It should be pointed out that the affinity of dibenzo-18-crown-6 for other metals ions, for example Ba$^{2+}$ and Ca$^{2+}$, is much less than for Na$^+$. Also, for these two particular multiply charged metal ions, 2:1 and 3:1 crown:metal complexes are observed in the ES spectrum rather than the 1:1 complex. However, formation of a 1:1 complex for most metals is probably possible since a large number of crown ethers have been synthesized that are selective for specific metal ions or sets of metal ions.

The selective nature of crowns for particular metal ions can be used to selectively extract metal ions from aqueous solutions into an organic solvent for analysis by ES-MS. The spectrum in Figure 4 was obtained from the toluene extract of a water/HNO$_3$ solution containing each of the alkali metals using 12-crown-4 as the complexing agent. The peaks indicative of several of the metals present in the water solution, namely peaks from the Li-, Na-, and K-crown complexes, are observed. It should be pointed out that no signal from the metal ions could be observed when spraying the water/HNO$_3$ solution. The spectrum obtained from the extract is complicated by the presence of H$_2$O$^+$, H$_2$O$_2^-$, and MeOH$^+$-crown complexes. However, this problem might be alleviated by adjustment of solution pH. More importantly, the Ca- and Mg-complexes are not observed since the affinity of 12-crown-4 for these ions is very low. Other crowns could be used to selectively extract these metal ions versus the other alkali metals in the mixture.

We have also begun to investigate the MS/MS behavior of the metal-crown complexes. Initial results indicate that certain of the 1:1 metal-crown complexes dissociate by loss of the neutral crown with the charge remaining with the metal. Results for complexes where the crown:metal ratio was greater than 1:1 show successive losses of the neutral crowns from the metal. Based on these results, it appears that MS/MS spectra of a series of metal-crown complexes using a single crown and/or a single metal with several different crowns might be used to determine the relative gas-phase affinities of selected metals and selected crowns. Also, the data from the MS/MS spectra might be used to determine successive metal ion-crown bond energies.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics


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ANALYSIS OF SOME POLY-PHOSPHONIC ACIDS BY IONSPRAY/MS

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ISP (Ionspray) is a version of ESP (Electrospray) in which droplets are created by both a high electric field and pneumatic nebulization. Both have recently been regarded as powerful soft ionization techniques for characterization of very high molecular weight biopolymers and many polar, thermally labile compounds. In this study, it is found that polyphosphonic acids especially with polyamine backbones are very suitable for such ionization techniques.

ISP/MS

Four compounds of X-(PO₃H₂)x, Structures I-IV, were used for this study. None yielded TSP spectra (positive nor negative). Static FAB* showed distinct MH⁺ and MₓMH⁺ in the (+) and M⁻H⁻, MₓM⁻H⁻ in the (-) modes for I and II but none for III and IV when X is a polyamine. Ionspray showed very distinct molecular ion information in both modes for I, II and IV. Only negative spectra are useful for III. In general, the negative spectra are overall more sensitive. 1 ng of I by flow injection mode gave a structurally recognizable spectrum in the (-) mode as shown in Fig. 1.

MS/MS

Daughter scans of either the MH⁺ or (M-H)⁻ of each compound yielded many different product ions by CID, as shown in Figs. 2 and 3.

From these experiments, Ionspray offers high ionization efficiency for this type of small and yet very polar compounds. Unambiguous molecular ion information are attainable through simple sample introduction. MS/MS experiments provide additional qualitative information through the product ions.

*Hutchinson, D. and Semple, G. Organic Mass Spectrometry, Vol. 20
No. 2 1985 143.
Fig 1
ISP/MS of Compound I (1 ng)

Fig. 2
Daughter Scan of m/z 300 of II

Fig 3
Daughter Scan of m/z 298 of II
Thermospray Interface Line Ammonolysis of Diethylamine Derivative of p-Phenylene Diisocyanate and Diethylene Glycol/Adipic Acid Polyester During LC/MS Analysis

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We have observed that ammonolysis of diethylamine derivatized phenyl isocyanate(A), dipropylamine derivatized phenyl isocyanate(B), and diethylamine derivatized p-phenylene diisocyanate(C) occurs in the thermospray interface line. Evidence for this ammonolysis reaction was obtained by the isolation and collection of compound C with the thermospray interface at ambient, 200, 250, 300, and 350 °C.

The data also suggests diethylamine(dipropylamine) liberated during the ammonolysis becomes available for adduct formation.

Hydrolysis of diethylene glycol/adipic acid polyester was observed in the mass spectra resulting from thermospray LC/MS. LC/MS/MS analysis was also performed on the polyester sample.

The thermospray mass spectra observed for compounds A, B, C, and a polyester were more complex than expected. Ions were observed that only could be explained as coming from multiple compounds based on protonated/ammoniated pairs (see Figures 1 & 2).

Compound C was separated by LC and collected from the thermospray interface line at ambient, 200, 250, 300, and 350 °C into a Reactiva® cooled with ice water. Only one LC peak representing compound C was found from the ambient temperature collection, whereas at 300 °C there were at least four new compounds formed. The mass spectra corresponding to these newly formed compounds suggests products of an ammonolysis reaction (see Figure 1). The mass spectra were very complex because each LC peak contained several ammonolysis product compounds as well as possible adduct ions of the various compounds (see Figure 2).

Figure 1. Ammonolysis Scheme and Thermospray Ions
The relative amounts of the major compounds (detected by UV) obtained by collecting compound C at various temperatures are shown in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>200 °C</th>
<th>250 °C</th>
<th>300 °C</th>
<th>350 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound C</td>
<td>100 %</td>
<td>52 %</td>
<td>11 %</td>
<td>1 %</td>
</tr>
<tr>
<td>LC peak 4</td>
<td>9 %</td>
<td>43 %</td>
<td>25 %</td>
<td>25 %</td>
</tr>
<tr>
<td>LC peak 2</td>
<td>9 %</td>
<td>6 %</td>
<td>63 %</td>
<td>74 %</td>
</tr>
</tbody>
</table>

Analogous to the ammonolysis effect, hydrolysis was also observed in the thermospray interface line for a diethylene glycol/adipic acid polyester. Some of the LC peaks were found to contain a series of ammoniated ions associated with small oligomers of the polyester. The presence of these ions can best be explained as hydrolysis products formed during passage of the polyester through the Interface line. The thermospray mass spectrum of the polyester gave several ions including protonated, ammoniated ion pairs which can be assigned structures of various polyester oligomers (see Figure 3).

LC/MS/MS experiments on ions at 772, 666, 556, 450, and 234 daltons yielded fragmentation ions expected for the diethylene glycol/adipic acid polyester:
Overview: Thermospray mass spectrometry (TSMS) combined with high-performance liquid chromatography (HPLC) can analyze for steroids without the need for derivatization (1). Because TSMS can be used with polar solvents and electrolytes, an electrochemical (EC) cell can be conveniently attached (2). During the HPLC/TSMS analysis of the steroid oxymetholone, concern developed with respect to the ease of oxidation of this compound and its possible metabolic fate. The electrochemical oxidation of oxymetholone was investigated by a combined EC/TSMS technique referred to as solution electrochemistry mass spectrometry (SECYMS).

Experimental: A Vestec Thermospray controller and source was attached to a Finnigan 4500 quadrupole mass spectrometer. A Coulochem 5100A potentiostat and model 5020 coulometric guard cell were interfaced to the mass spectrometer. The liquid carrier for direct injections consisted of 50/50 methanol (MeOH)/water with 0.1 M ammonium formate. The mobile phase for HPLC was comprised of 85/15 MeOH/water with 0.1% acetic acid. Nominal concentrations of steroids diluted in MeOH or acetonitrile (ACN) were 0.02 mg/ml with 100 μL injections. HPLC with an ultraviolet (UV) detector was set at 280 nm. Figure 1 shows the block diagram for direct injections and HPLC analyses.

Results and Discussion: The TSMS of oxymetholone produces an [M + H]+ ion at m/z 333 with no fragmentation. Similarly, d2-testosterone gave an ion at m/z 291. Because of chemical modification of oxymetholone diluted in methanol (3), steroids were diluted in ACN. Oxymetholone was analyzed by the SECYMS method via direct injection over a range of 0.0 to 0.8 V vs. SHE (Figure 2). At a potential of approximately +0.6 V, the oxymetholone oxidized to a compound with an ion at m/z 319. Figure 3 demonstrates the changes before and after oxidation for oxymetholone. A similar investigation of d2-testosterone gave no indication of oxidation.

An EC/HPLC/UV/TSMS experiment was performed to observe the retention time of the compound produced in the SECYMS experiment. On injection followed by oxidation, the chromatographic peak of oxymetholone (6.7 min) diminished and a new chromatographic peak at approximately 3.6 min was observed (Figure 4) with an ion at m/z 319.

Conclusions: The SECYMS experiment clearly demonstrates the ease of oxidation of oxymetholone compared to the similar steroid d2-testosterone. In comparing these two molecules (Figure 5), a major difference is the vinyl alcohol group attached to the 2-position of oxymetholone. It is speculated that this vinyl alcohol substituent is being affected during electrochemical oxidation. This is substantiated by comparison of the chromatographic peaks in Figure 4. Note that the oxymetholone has a broadened peak compared to the compound eluting at 3.6 min. It has been reported that the intramolecular hydrogen bonding of the vinyl alcohol group results in oxymetholone adsorbing to various surfaces such as alumina (4). With oxidation of the vinyl alcohol group, this "stickiness" disappears for the compound eluting at 3.6 min. The compound represented by the ion at m/z 319 may be the result of a carbonyl replacing the =CHOH substituent in oxymetholone. It was also observed that the compound eluting at 3.6 min did not produce a detectable UV signal at 280 nm.

This study suggests that oxymetholone, unlike the majority of other steroids, may be readily altered by in vivo processes that can result in the oxidation of oxymetholone to form metabolites with little of the parent oxymetholone remaining. Future work will involve a comparison of compounds isolated from biological systems to those observed in this study.

References:

The authors wish to acknowledge the kind gift of steroids from Dr. Tom Goehl, National Toxicology Program.
Figure 1

Figure 2

Figure 3

Figure 4

Figure 5
INTRODUCTION: Oligomycins A, B and C are of biochemical interest mainly because of their use as ATP-ase inhibitors. These oligomycins were first isolated in 1954 from a strain of *Streptomyces diastatochromogenes*. The structures of oligomycins have been determined by a variety of techniques including X-ray crystallography as well as spectroscopic and chemical correlations. Oligomycins are neutral macrolide antibiotics containing a 26-membered α,β-unsaturated lactone with a conjugated diene, fused to a bicyclic spiroketal. The EIMS of these compounds and their derivatives have been reported.

We have investigated the use of negative ion thermospray (NTSP) LC/MS for the qualitative analysis of oligomycins. To study the fragmentation behavior of the compounds under thermospray MS conditions, we have used the commercially available oligomycins (A,B,C), as well as a recently described new compound F28249o, as models. The structures of oligomycins are shown in Scheme 1.

EXPERIMENTAL: LC/MS was performed on the Finnigan MAT TSP-46 single quadrupole mass spectrometer with a Finnigan thermospray interface. The thermospray spectra were obtained in the negative ion mode using the thermospray interface operated at a jet temperature of 230 °C, Finnigan sapphire vaporizer (temperature range 70 to 100 °C), a repeller setting of 1KV and the discharge ionization electrode at 1 KV, mass range 150-900 amu, 1.5 sec/scan rate.

The HPLC system consisted of a Waters 600-MS solvent delivery system and WISP 710B autosampler with 990-MS photodiode array detector. The oligomycins were separated on a Waters C-18 Nova-Pak column(5μ, 150mm x 3.9mm ID). The mobile phase was 75% methanol in water and/or 0.1M ammonium acetate (pH=6.5) buffer at a flow rate of 1.2 ml/min. The detection was by UV absorbance at 225 nm.

RESULTS & DISCUSSION: Thermospray MS has been classified, as a soft ionization technique primarily yielding molecular weight information. This information is not sufficient for structure determination of unknown or even suspected known compounds. However, by controlling the TSP-MS parameters such as vaporizer temperature, buffer ionization, repeller voltage and the mode of ionization (positive ion or negative ion), it is possible to obtain molecular weight plus structural information for the determination of structures of unknown compounds. The objective of the present study was to extend the analytical capabilities of the LC/TSP-MS technique with a single quadrupole instrument.

Negative ion TSP-MS: Oligomycins showed better sensitivity in the negative ion mode than in the positive ion mode under LC/TSP-MS conditions. Negative ion TSP spectra showed electron capture molecular ions [M-], [M-H]-, [M-H2O]-, and abundant thermally induced fragment ions. The genesis of fragment ions is due to three types of fragmentations: retro-aldol, ester cleavage and retro Diels Alder. These ions are diagnostic for the characterization of oligomycins (Scheme 1).

The [M-H2O]- ion (F1) is more abundant than the M-. in A,B, & F28249o, while the M-. ion is dominant in oligomycin-C. Retro-aldol (RA) fragment ions (F2) at m/z 574, 588, 574, and 590 are dominant in the NTSP-MS of oligomycins A, B,C & F28249o respectively. The mass shift of RA ions is the indication of structural modifications in the top portion of the molecule. Similarly, the ions observed at m/z 214(F4) and 196 (F5) in A, B and F28249o, indicate the presence of an OH group at C-12. The ion at m/z 296(F12) observed in NTSP-MS of oligomycin-F28249o, indicates the presence of an OH group at C-21. NTSP-MS of oligomycin-B showed a diagnostic ion at m/z 281(F10). NTSP-MS of oligomycins
showed the formation of adduct ions [M+CH3COOH]-, and [Fragment ion F4 +CH3COOH]-, under ammonium acetate buffer ionization conditions.

**Mixture analysis of Oligomycins.** A standard mixture of oligomycins-A, B, C & F28249ω (2 g/L) was prepared and 50 μL injected for HPLC/UV (225nm) and LCMS analysis. The compounds were well resolved with 75%MeOH in ammonium acetate buffer. The elution order of the compounds is F28249ω (7.5min), oligomycin- B(8.94min), A(15.14min) and C(24.55min). The crude extract of F28249 was also analyzed using LC/NTSP-MS under similar conditions. This sample showed four peaks, the major compound (88%) is F28249ω, whereas the other three minor compounds ω2(M-. 792 ), ω3(M-. 820), & ω4(M-. 790) are unknown. Based on the LCMS and UV data the structure of F28249ω has been proposed as shown in Scheme 1 (F28249ω: R1= H2, R2= H, R3= OH).

**CONCLUSIONS:** We have successfully demonstrated here, that the combined LC/UV and LC/TSP-MS are powerful tools for the direct analysis of fermentation products for detection and identification, as well as structure determination of unknown oligomycins. The present work is another example where negative ion is superior to the positive ion TSP-MS.

**REFERENCES:**
A mixture of eight selected azo and quinone dyes was analyzed using a Hewlett-Packard thermospray (TS) ion source. The selected dyes are shown in Table I. Azo and quinone dyes are used for textile dyeing and are commonly found in waste water, soils and waste materials in and near textile processing and manufacturing. Most azo dyes cannot be analyzed by gas chromatography-mass spectrometry because of their thermal lability. Recent methods for analysis of the selected set of dyes, provided a method which used a 2.1 mm LC column using a 0.4 mL/min flow rate coupled to a down stream "Tee" with 0.8 mL/min ammonium acetate buffer for post column addition. The post column addition requires additional plumbing and an additional HPLC pump to execute the method. Similar chromatographic separations of the selected dyes may be obtained by the use of either a 4.6 or a 2.1 mm column incorporating the buffer in the precolumn configuration.

The Thermospray signal response has a strong dependency on both the buffer concentration and more importantly, the TS ion source pressure. Given the buffer to be held at a constant concentration, the ion source pressure becomes the important factor. Maintaining the pressure in the TS ion source can be accomplished by increasing the LC flow rate or by throttling the TS ion source pump out valve when flow rates under 1.0 mL/min are required for chromatographic separation. The throttling technique may be used for flow rates down to 0.2 mL/min.

The ion source used with the dyes was a standard HP ion source incorporating a fragmentor electrode which enhanced the sensitivity up to a factor of 20 when optimized near 55 volts. The ion source is shown in Figure 1.

TABLE I

| DISPERSE BLUE 3 | DISPERSE ORANGE 3 |
| DISPERSE YELLOW 5 | DISPERSE BLUE 14 |
| DISPERSE RED 1 | SOLVENT RED 23 |
| DISPERSE BROWN 1 | DISPERSE RED 13 |

The fragmentor electrode provides increased sensitivity for all dyes in this analysis. By increasing the voltage from 55 to 200 volts, the dyes were fragmented in to smaller ion fragments. The fragmentation appears to be due to the acceleration of electrons and ions in the TS source near the ion exit cone.

Figure 2 shows the chromatogram of the eight dyes with many impurities, homologues and isomers of the dye set. The LC conditions were: A = 0.05 M NH4OAc and B = MeOH. Linear gradient from 50% B to 90% in 13 min. on a 4.6 x 120 mm C-18 3um column without post column addition. Flow rate was 1.0 mL/min.
Figure 1. TS ion source with Fragmentor

Figure 2. Azo dyes by thermospray LC/MS
In the past decade, the first reports of the acquired Immunodeficiency syndrome (AIDS) surfaced, the human Immunodeficiency virus (HIV) was discovered to be the causative agent, and 3'-azido-2',3'-di-deoxythymidine (AZT) was approved as an AIDS-static agent. A large percentage of the compounds which have been shown to display activity against HIV in vitro are 2',3'-dideoxynucleosides [2] or 2',3'-dehydro-2',3'-dideoxynucleosides [3]. In addition, C-Nucleosides have been shown to possess a variety of biological activity, most importantly as potential anti-tumor and anti-AIDS agents (e.g., showdomycin [4]).

In pursuit of new routes to such compounds, (i.e., 2' or 3' modifications) one should include the possibility of utilizing the corresponding 2' or 3'-keto derivatives. Though these keto nucleosides have been available since the 1960's, little has been reported on their use in the synthesis of nucleoside analogues. This is largely because keto nucleosides are perceived to be unstable, especially under basic conditions. Similarly, little effort has been reported on synthesis of nucleosides via the corresponding oxyglycals. We have recently discovered conditions which allow palladium(0) catalysts to undergo oxidative-addition with 2,3,4,6-tetrabenzylglycosyl methanesulfonate (mesylate), followed by rapid beta-hydride elimination. This method offers a two-step, one-pot route to the synthesis of (perbenzyl)oxyglycals.

In pursuit of new routes to such substituted sugar analogues, a substantial amount of oxyglycal, 3, was required. Benzylatlon of methyl glucoside affords 2 [5]. After hydrolysis [5] and generation of the mesylate [6], oxidative-addition of palladium, followed by beta-hydride elimination affords 3. In general, NMR spectra of these compounds are complex and not always definitive, therefore we have sought additional means of analysis. In addition, the mesylate is relatively unstable, therefore, a practical method of following the progress of these reactions is required. Thermospray LC/MS has proven ideal in this regard. In this study we report on the LC/MS/MS analysis of oxyglycals produced via the palladium catalyzed reaction shown in Figure 1.

![Diagram of reactions used to produce tetrabenzyl oxyglucal from alpha-methyl glucoside](image)

A VG Trio 3 triple quadrupole (with a hexapole collision cell) mass spectrometer was used in this study. All compounds were stable and separable under HPLC conditions using a Waters 600 MS HPLC system with a 25 cm x 4.6 mm reverse phase C18 column. In order to minimize the time of exposure of these compounds to water, 100 percent acetonitrile was used as the mobile phase (0.7 mL/min) and the electrolyte solution for thermospray, 0.1 M ammonium acetate (adjusted to pH 5 with acetic acid), was added post-column (using an ISCO 2350 HPLC pump; 0.5 mL/min) prior to introduction to the mass spectrometer. In all cases, a molecular ion of the type [M + NH₄]⁺ was observed as the base peak in the thermospray mass spectrum. Once observed, the molecular ion was mass selected in quadrupole one, collisionally activated in the hexapole collision cell using xenon gas at 1 x 10⁻⁵ mBar analyzer pressure (normal operation pressure ca. 3 x 10⁻⁸ mBar) and 5 eV collision energy.

The MS/MS spectra of the glucose analogues contain virtually the same fragment ions. In the MS/MS spectrum of methyl (perbenzyl) glucoside, a fragment ion at m/z 523 is observed and is assigned as loss of ammonia and methanol leading to the formation of an oxyglucal cation species which goes on to lose benzyl alcohol forming a fragment ion at m/z 415. This fragment ion, as well as all others that follow are common to all MS/MS spectra shown in Figure 2. In the MS/MS spectrum of (perbenzyl) glucose, fragment ions at m/z 540 and 523 are observed and assigned to loss of
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water and loss of ammonia, respectively, again leading to the formation of an oxyglucal cation species. It is not surprising, therefore, that the \([M + \text{NH}_4]^+\) molecular ion of (perbenzyl) oxyglucal would lose ammonia and benzyl alcohol to form the fragment ion at m/z 415. This result suggests that each ion goes through its most stable gas phase configuration, that of the oxyglucal, prior to eliminating benzyl alcohol.

In order to be of use in nucleoside synthesis, the reactions were performed on ribose. In general, the tribenzyl ribose and tribenzyl ribul produced abundant molecular ions of the type \([M + \text{NH}_4]^+\) (see Figure 3). Thermospray MS/MS of these compounds produced spectra which contained (as above) structurally significant fragment ions corresponding to the known structure of each molecule. For example, in the MS/MS spectrum of (perbenzyl) ribose fragments ions at m/z 420, 403, and 295 are observed corresponding to loss of water, then loss of ammonia followed by loss of benzyl alcohol, respectively. Fragment ions at m/z 181 and 193 are formed via ring cleavage reactions. It is interesting to note that an ion at m/z 193 is not observed in the MS/MS spectrum for (perbenzyl) ribul. It is possible that the double bond in the ring prevents this product ion from forming.

Figure 2. Thermospray MS/MS spectra of perbenzyl... a) oxyglucal; b) methyl glucoside; c) oxyribul; d) ribose.

Recently, Danishefsky reported on a method for the stereoselective epoxidation of glycals using dimethyldioxirane [8]. Danishefsky further reported that the epoxides formed underwent clean ring opening at the anomeric carbon when using \(\text{O}-\text{nucleophiles}\) [7]. In future experiments, it is hoped that through epoxidation of perbenzyl ribose using the technique reported by Danishefsky, an easier route will be discovered towards the formation of 2'-ketonucleosides.

Many species of higher fungi produce specially adapted propagules called sclerotia as a means of surviving harsh climates or nutrient-poor conditions (2). These relatively large resting bodies can survive for several months to several years in the soil, despite exposure to insects that consume fungi. Our studies have shown that sclerotia produced by Aspergillus species frequently contain antinsectan metabolites, some of which deter feeding by fungivorous insects (2,3).

In this paper, the results of thermospray mass spectrometric analysis of a chloroform extract from a non-aflatoxigenic strain of Aspergillus flavus are presented. The characterization of metabolites from these extracts originally involved collection of chromatographic fractions followed by further purification and analysis using NMR and mass spectrometry. Of ten compounds isolated from a chloroform extract of A. flavus sclerotia using HPLC, four were shown to have potent antifeedant activity against fungivorous insects which encounter sclerotia in nature. Three of these had not been previously reported (2,3).

A VG Trio 3 triple quadrupole mass spectrometer was used in this study. All compounds were stable under HPLC conditions using a Waters 600-MS HPLC system with a 25 cm x 4.6 mm reverse phase C18 column. Since the mobile phase consisted of 70:30 methanol:water, the 0.1 M ammonium acetate electrolyte solution (adjusted to pH 5 with acetic acid) necessary for thermospray ionization was added post-column. This provided a relatively simple means of assuring significant and stable levels of ionizing reagent for ion production. The column flow rate was 0.7 mL/min. The electrolyte solution for thermospray was added post-column (after the UV detector) using an ISCO 2350 HPLC pump at a flow rate of 0.5 mL/min prior to introduction to the mass spectrometer. In all cases, a molecular ion of the type [M + H]+ was observed, however, the [M + NH4]+ molecular ion was the base peak of the mass spectrum, In most cases. A VG 11-250 J data system coupled to a DEC PDP 11/53 was used to acquire data. The UV output was collected simultaneously with the total ion chromatogram (Figure 1). A 10- to 15-second delay occurred between the UV and TIC peak onsets.

Figure 1 Total ion current chromatogram and UV trace (215 nm) from the thermospray analysis of the chloroform extract from the sclerotia of Aspergillus flavus

Several compounds have been previously isolated and characterized from the chloroform extract of the sclerotia of Aspergillus flavus (see structures 1-6 in Figure 2). In the experiments reported here, four UV active chromatographic peaks of significant abundance were observed (see Figure 1). The first peak was characterized as having molecular ions at m/z 455 and 472 corresponding to [M + H]+ and [M + NH4]+ respectively (see Figure 2a). Characteristic of most thermospray spectra, fragment ions in general were of low abundance or not observed. One fragment ion observed was at m/z 440 corresponding to loss of methyl from the protonated molecular ion or loss of methane and ammonia from the ammoniated molecular ion.

Peak two was characterized by the molecular ions at m/z 438 and 455 ([M + H]+ and [M + NH4]+), respectively; Figure 2b). Fragment ions at m/z 420 and 403 were observed and assigned as consecutive loss of water and OH from the protonated molecular ion.

Peak three had previously been separated into two components (3 & 4) using a 25 cm x 10 mm; semi-preparative column at 2 mL/min. The structures of compounds 3 and 4 are shown in Figure 2c. The complex nature of the molecular ion region is characteristic of the mixture present in the peak (see Figure 3 for mass chromatogram overlay of these two compounds). For example, the ions at m/z 418 and 435 are assigned as the [M + H]+ and [M + NH4]+ molecular ions, respectively, of compound 3. Fragment ions are observed at m/z 400 and 383 corresponding to loss of water and of water and OH, respectively. The ions at 422 and 439 are assigned as the [M + 2H]+ and [M + NH4]+ molecular ions, respectively, of compound 4; a fragment ion at m/z 404 is observed as loss of water from the protonated molecular ion.

Peak three had previously been separated into two components (3 & 4) using a 25 cm x 10 mm; semi-preparative column at 2 mL/min. The structures of compounds 3 and 4 are shown in Figure 2c. The complex nature of the molecular ion region is characteristic of the mixture present in the peak (see Figure 3 for mass chromatogram overlay of these two compounds). For example, the ions at m/z 418 and 435 are assigned as the [M + H]+ and [M + NH4]+ molecular ions, respectively, of compound 3. Fragment ions are observed at m/z 400 and 383 corresponding to loss of water and of water and OH, respectively. The ions at 422 and 439 are assigned as the [M + 2H]+ and [M + NH4]+ molecular ions, respectively, of compound 4; a fragment ion at m/z 404 is observed as loss of water from the protonated molecular ion.
Figure 2. Thermospray mass spectra from the four major UV active chromatographic peaks observed.

The fourth peak, much like peak three, is a mixture of two compounds. In this case, the compounds are isomers of molecular weight 421 (see Figure 2d). The molecular ions at m/z 422 and 439 are observed, with fragment ions at m/z 404 and 386 assigned to two consecutive losses of water from the protonated molecular ion. A molecular ion of the type \([M + NH_3 + NH_4]^+\) is also observed at m/z 456. The reason for reconvolution of the last two chromatographic peaks is at this time unknown.

This technique will provide for a more streamlined approach toward the surveying of sclerotial extracts for unique bioactive metabolites. It is clear that improvements in both chromatographic resolution and ion sensitivity will be necessary to make this technique analytically useful. In the case where co-eluting compounds of different mass are observed, MS/MS experiments will be performed to enhance the structurally significant fragment ions observed.

INVESTIGATION OF EIGHT HETEROCYCLIC AMINES AND TWO OF THEIR N-OXIDATION PRODUCTS BY THERMOSPRAY MASS SPECTROMETRY (TSMS)

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INTRODUCTION

The formation of mutagenic polycyclic aromatic hydrocarbons (PAHs) during the cooking process of foods, primarily meat products, was reported by Lijinsky and Shubik in 1964 (1). The development of the Ames assay in 1975 made it possible to test food products for mutagenic activity, then isolate new mutagenic compounds formed in these products. Several years later, Sugimura and co-workers (2) reported that the mutagenic activity of cooked fish and beef products could not be accounted for by PAHs alone. This resulted in the discovery of the aromatic heterocyclic amines as the group of compounds responsible for this additional mutagenic activity (3). Some of these heterocyclic amines have been found to be suspected carcinogens (4). These findings substantiate the need to develop sensitive analytical techniques to detect these compounds in various cooked meat products at the ppb level. Yamaizumi et al. (5) have reported the use of high-performance liquid chromatography/thermospray mass spectrometry (HPLC/TSMS) for the analysis of two of these compounds. We now report the analysis of eight of these heterocyclic amines by HPLC/TSMS plus the synthesis and analysis of two of their N-oxide derivatives.

EXPERIMENTAL

HPLC/TSMS system: The eight heterocyclic amines and two of their N-oxidation products were resolved using a SynChropak SCD-100 column (Synchrom, Inc., Lafayette, IN). The silica base packing was 5 microns (particle size) with 100 angstrom average pore size. The mobile phase was 0.05 M ammonium acetate in water adjusted to a pH of 5.2 with acetic acid/acetonitrile (75/25, v/v). An Isco (Lincoln, NE) model LC-5000 syringe pump was used at a flow rate of 1 ml/min connected to a Rheodyne 7125 injector fitted with a 20 μl loop. TSMS analysis was performed using a Delsi Nermag (Houston, TX) R1010C quadrupole mass spectrometer equipped with a Delsi/Nermag TS source and a Vestec (Houston, TX) TS interface and controller. The TS source was operated in the positive ion, "discharge off" and "filament off" mode. Typically, the TS source block was set to 160°C and the control temperature was set at 132°C.

NMR: 1H NMR spectra of IQ, MeIQx and their N-oxidation products were carried out at 500 MHz on a Bruker AM500 spectrometer. The chemical shifts are reported in ppm by assigning the DMSO resonance to 2.49 ppm. Spectral parameters are first order measurements.

Synthesis of N-oxides: The synthesis of the N-oxidation products were accomplished using the method of Chaudhuri et al. (6) for the preparation of antihistamine N-oxides, using the m-chloroperoxybenzoic acid method.
RESULTS AND DISCUSSION

We analyzed a series of eight heterocyclic amines by thermoSDrav mass spectrometry (TSMS). For each compound the [M+H]+ ion was the base peak. We also used TSMS to analyze the two synthesized N-oxidation products of IQ and MeIQx. As shown in Figure 1, the [M+H]+ ion for MeIQx was observed at m/z 214 as the base peak. The N-oxidation product of MeIQx had an [M+H]+ ion at m/z 230 (see Figure 2). The TS mass spectrum for IQ included the [M+H]+ ion at m/z 199 as the base peak, while for the IQ N-oxidation product the TS mass spectrum exhibited an [M+H]+ ion at m/z 215.

The NMR results confirmed the site of this oxidation as that of the N-6 location on the quinoline moiety. The oxidation product of MeIQx also resulted in a confirmed site of oxidation at the N-6 location on the quinoline moiety.

An investigation of the utility of the HPLC/TSMS method for the analysis of these chemicals as well as for compound identification of in vitro and in vivo metabolites is in progress. These mutagenic aminoimidazo azaarenes will be assayed in samples derived from both cooked meat products and cigarette smoke condensate using the HPLC/TSMS method.

REFERENCES

We have carried out a careful study of the ionization efficiency in ionspary (pneumatically assisted electrospray) mass spectrometry [1]. Not only ion currents arriving at the detector of the mass spectrometer were measured, but also the current leaving the electrospray/ionspray device in the atmospheric pressure ion source. Spray currents and ion currents are conveniently measured as a function of sample concentration by flow injection with a large sample loop (50 µL) which delivers a broad sample plug at a low flow rate (5 µL/min) into the electrospray/ionspray device. The measured currents can be backgound subtracted to obtain "true" sample spray currents and ion signals.

We have found that the base spray current of a "pure" HPLC quality solvent is apparently due to salts present as impurities. In a particular solvent, the steady state concentration of salts arriving at the sprayer is the result of the equilibrium between adsorption and desorption from components in the liquid handling system. When a strongly solvating solvent (100% methanol) is injected into a stream of solvent having a lower polarity (95% acetonitrile + 5% methanol), sodium salts are released from the walls of the liquid handling system and produce anomalous spray currents (Figure 1) and anomalous sodium adduct ion signals of neutral components in the liquid phase or the gas phase in the atmospheric pressure ion source. When a less polar solvent mixture (95% acetonitrile + 5% methanol) is injected into a stream of a strongly solvating solvent (100% methanol) a temporary decrease of the spray current (and XNa+ ion signals) followed by a short peak is observed (Figure 2). A true "blank" solvent injection and reliable sample injections can be performed only if the solvent stream and the solvent used for the sample solution are identical and preferably taken from the same container.

Sample adsorption problems are usually associated with chromatographic columns. Adsorption phenomena can also interfere with flow injection experiments. We found for example that 8-hydroxyquinoline glucuronide is strongly adsorbed and slowly desorbed when injected as a solution in 95% acetonitrile + 5% methanol. The addition of acetic acid to a final concentration of 10^-4 M alleviates the adsorption problem. The wall of the fused silica transfer line between the injector and the ionspary nebulizer appears to be the major adsorption site.

The mass spectrometer used is a NERMAG R3010 triple quadrupole equipped with a home made atmospheric pressure ion source and ionspray LC/MS interface

INTRODUCTION:
The combination of capillary electrophoresis (CE) and ion-spray mass-spectrometry provides a highly efficient technique with very selective detection. We previously reported this coupling via a liquid-junction interface. We now describe several design modifications which improve its analytical ruggedness and overall performance. Gel capillary electrophoresis represents another field of growing interest. We have challenged the liquid-junction ion-spray interface to also achieve gel CE/MS coupling.

EXPERIMENTAL:
Capillary electrophoresis was performed on an in-house constructed CE unit, and a Beckman P/ACE 2000. We coupled it to a TGA 6000E (upgraded to an API III) triple-quadrupole mass-spectrometer equipped with an API source. The interfacing was performed by means of a liquid-Junction (1). The improved version is presented on fig. 1. Free-zone CE separations were performed in 75 µm i.d. untreated fused-silica capillaries. Gel CE separations were accomplished in gel-filled capillaries from ABI (Micro-Gel 100 50 µm i.d. x 40 cm) and Beckman (Experimental formulation 75 µm i.d. x 55 cm). The inlet buffer was used as provided by the gel manufacturer (ABI: 75 mM Phosphate, 10% MeOH; Beckman: 100 mM Tris-Borate, 7M Urea) while the make-up buffer was 25%ACN, 5% Formic Acid for positive ion detection and 20%MeOH, 0.1% Triethylamine for negative ion detection.

RESULTS & DISCUSSION:
By using short columns and high electrical fields (1000 V/cm), one is able to achieve “fast” separations. We analysed a mixture of eight sulfonylureas (environmentally important herbicides) in less than 6 minutes in the 20 pmol range in the full scan mode (fig. 2). We have also been able to accomplish on-line CE/MS/MS analysis providing helpful assessment of the structure of two isobaric analytes since one of them doesn’t yield the benzylic cleavage.

Our first attempt to couple gel CE with mass spectrometry showed some promising aspects. However, the low sample capacity of these columns required operation of the mass spectrometer in the Selected Ion Monitoring (SIM) mode. Fig. 3 shows a gel-CE/SIM-MS analysis of a sample of polyacrylic acid. The most abundant ions in the sample (doubly-charged anions) were determined by infusion experiments of the sample diluted in the make-up buffer and then monitored by SIM during gel-CE/MS analysis. In spite of the low electrophoretic resolution, one can establish part of the given polymeric distribution of this sample.

CONCLUSION:
We were able to realize full-scan CE/MS analysis of a mixture containing eight sulfonylureas (herbicides) in less than 6 minutes. On-line CE/MS/MS provided sufficient information to characterize two isobaric sulfonylureas. We have accomplished the coupling of gel capillary electrophoresis with mass spectrometry and thus demonstrated the feasibility of this approach.
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1. Mounting Screws (Nylon)
2. Capillary Holder (Plungers)
3. Liquid-Junction Block (PEEK)
4. Support (plungers) mounted on a X-Y-Z positioner
5. Transfer line: 75 μm x 375 μm
6. Capillary guides (PEEK tubing cut out of Upchurch capillary sleeves)
7. CE column: 75 μm x 375 μm
8. Slip-on Union

Fig. 1 - Liquid-Junction Interface

Fig. 2 - Fast CE/MS analysis of a synthetic mixture of sulfonylureas

Fig. 3 - Gel CE/SIM-MS analysis of a polymeric distribution of polyacrylic acid
Investigation of CAD Process and CAD Spectra Generated in the Transport Region of an Electrospray Interface

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Introduction
Electrospray MS has the capability to efficiently generate ions from a wide variety of compounds down to the low pg level. While the sensitivity obtained with electrospray MS is excellent, structural information is often non-existent. Usually only [M+H]+ and/or adduct ions and multiply charged ions are detected due to the gentle nature of ionization. Often the more costly combination of ES with MS/MS is employed to gather structural information on compounds of interest.

This paper reports on the use of collisional activation (CA) in the transport region of an electrospray single quadrupole MS as a way to obtain structural information for some environmentally and biologically significant compounds. Collisonal activation in the transport region can occur between the end of the capillary and the first skimmer (skimmer 1) by controlling the potential differences between these two points to accelerate ions in the high pressure supersonic expansion region. The ability to generate interpretable CAD spectra in the transport region of the electrospray interface could have significant ramifications in environmental and biological monitoring.

Experimental
The electrospray source (Analytica of Branford, Branford, CT) was interfaced to a Finnigan MAT 4500 Quadrupole MS (San Jose, CA). The ES design was similar to that reported previously [1]. The samples (10 pg-100 mg dissolved in 50% methanol in water) were introduced into the ES source through a 75 um i.d. tube at a flow rate of 2 uL/min using a syringe pump (ISCO Inc., Lincoln, NE) by means of a low pressure injection valve with a 5 uL loop (Analytica of Branford). The electrospray needle was held between +2.9 and 3.4 KV. A nitrogen drying gas heated to 185-190°C was introduced against the needle flow at a rate of 0.2-0.3 L/min. Positive ions formed from in the spray migrated down the 13.4 cm (0.5 mm i.d.) glass capillary which was positioned about 1.7-1.5 cm from the needle exit. Each end of the glass capillary was coated with a conductive metal to enable control of the electrical potential. The entrance of the capillary was grounded, but the exit voltage could be varied from 0-400 V to control collision energy. Ions migrated from the capillary through the 1.2 mm i.d. skimmer 1 typically set at 24 V (positioned 4.0 mm beyond the capillary exit) through skimmer 2 set at 17 V, then through a set of 3 lenses set at -21, 4.4 and -58 V respectively and finally into the quadrupole mass analyzer. The quadrupole was scanned from 10-500 daltons in four seconds. Pressure in the system ranged from atmospheric at the needle exit to 0.9 torr at skimmer 1 and 6 x 10^-6 torr in the quadrupole analyzer.

Results and Discussion
To determine the extent collision energy varied with the potential difference on the capillary and skimmer, internal energy distribution (P(E)) and average internal energy (Eaverage) of ions can be measured in the electrospray transport region in a similar fashion that has been reported for high and low energy collision in sector, quadrupole and ion trap MS/MS instruments [2,3]. Tetraethyl silane was used to estimate P(E) for various capillary-skimmer 1 potential differences shown in Figure 1A. The maximum internal energy (Emax) after a single collision was calculated and used as a reference in comparing the observed Emax values from the P(E) curve in Figure 2A. Emax of an ion after a single collision is given by energy for the center of mass (Ecom) plus the energy for the lowest energy fragmentation process (Eo). These observations (Figure 1A) support that (1) Emax can be used to estimate maximum internal energy, (2) that the width of the P(E) curve increases when increasing the potential difference between the capillary and skimmer, and most importantly, (3) the internal energy is proportional to the potential difference between the capillary and skimmer (Figure 1B).

The electrospray MS analysis of several pesticides and antibiotics demonstrates the capability of the system to generate interpretable CAD spectra as well as the capability to vary collision energy. For example, propoxur, a carbamate pesticide, exhibited an [M+H]+ ion at a capillary-skimmer potential difference of 25 V (Figure 2A). At a capillary-skimmer potential difference of 85 V fragments resulting from the loss of C3Hg, C3Hg and CONCH3, followed by loss of water are detected (Figure 2B). Potential differences above 140 V volts result in the formation of low mass fragment ions (e.g., m/z 314).
Furthermore, the CAD spectra for propoxur resulted in better than 70% daughter ion current yield compared to the non-CAD spectra. The major daughter ions for 10 pg of propoxur could be detected under full scan CAD conditions.

**Conclusions**

By varying the capillary-skimmer potential differences, $E_{\text{average}}$ from near 0 eV to greater than 7 eV can be selected (in a matter of seconds) for solving problems in structural elucidation and/or improved specificity. Furthermore, the operation at potential differences in the 0-200 V range did not change peak shape, instrument resolution, and resulted minimal loss in ion transmission.

**References**


**Acknowledgements**

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![Figure 1](image1.png)

**Figure 1.**

A) Potential energy distribution ($P(E)$) for various capillary-skimmer potential differences for the CAD of tetraethyl silane.
B) Average internal energy ($E_{\text{average}}$) acquired at various potential differences between the capillary and skimmer.

![Figure 2](image2.png)

**Figure 2.**

ES determination of 2 pg of propoxur in 1:1 MeOH:H$_2$O at a flow rate of 2 µL/min.
A) Spectrum of propoxur at a capillary-skimmer potential difference of 25 V
B) Spectrum of propoxur at a capillary-skimmer potential difference of 85 V
The electrospraying of aqueous solutions containing strong electrolytes such as trifluoroacetic acid (TFA), has, in the past proven difficult because of the high solution conductivities and similarly high surface tensions prevent the formation of a stable spray, as the applied voltages necessary to electrospray these type of solutions often produce a corona discharge in the surrounding air, and has consequently resulted in poor spray characteristics, ion current instability, and loss of sensitivity, which in electrospray LC-MS studies which often use these type of solutions, analysis has been made difficult.

Improvements have been developed for a conventional electrospray source allowing the spraying of high aqueous content solutions with improved stability and enhanced sensitivity allowing the on line analysis of peptides and proteins providing both sequence and molecular weight information.

The improved stability has been affected with the use of a multilayered needle device which delivers a concentric liquid sheath and a focusing gas around a central analyte flow. The desired properties for a good sheath liquid are that it be miscible with water and other mobile phase solvents, that it has a boiling point above that of water, to prevent premature evaporation of the solvent, and that its surface tension is low relative to water, to aid in droplet formation. In positive ion electrospray it has been found that 2-methoxyethanol provides the best performance, not only in improved stability but also enhances the observed ion signal. Isopropanol has found the most utility as a sheath liquid in the negative ion mode. These two solvents also possess electrical conductivities that are slightly higher than other potential sheath liquids such as butanol and methanol, although the implication of this property to improved spray stability is not fully understood.

Sensitivity enhancements have been obtained by the addition of a tube lens in the viscous gas flow region situated between the capillary exit and the skimmer orifice in the electrospray source. The tube lens focuses ions in an expanding jet and shapes and intensifies the ion beam through the gas disturbance and into the skimmer orifice. The lens also causes the ion beam divergence angle after the skimmer to be narrower than on a system operating without the tube lens. The lens shapes the electric fields so that more ions are forced down the jet centerline, thus increasing the fraction of ions captured by the subsequent lens system and the mass analyser and has increased the observed ion signal by up to a factor of five.

The effects of the combination of the sheath liquids and the tube lens are shown in figure 1. This showing the respective improvements in signal with choice of sheath liquid and the benefits afforded by the tube lens.

Figure 2. shows the stability observed in an electrospray LC-MS analysis of a Lactoglobulin A tryptic digest before and after implementation of the 2-methoxyethanol sheath and the tube lens.
FIGURE 1

Effect of Tube Lens and Sheath Liquid on ESI Sensitivity

FIGURE 2

ESI/LC/MS Chromatograms Obtained Before and After Improvements

TUNABLE COHERENT VACUUM ULTRAVIOLET PHOTOIONIZATION OF ALIPHATIC COMPOUNDS

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INTRODUCTION: Coherent VUV photoionization has become a very useful technique for soft, universal ionization. Until now, work with coherent VUV photoionization has been limited to 118.2 nm radiation which is easily produced by frequency tripling the third harmonic of a Nd:YAG laser. By using rare gas mixtures for third harmonic generation it is possible to efficiently produce coherent VUV radiation from 118 to 130 nm. The generation and use of this radiation is discussed.

EXPERIMENTAL: Coherent VUV radiation is produced by frequency tripling UV radiation in rare gas mixtures. For third harmonic generation of 118 nm radiation the third harmonic of a Nd:YAG laser (355 nm) is focused in a phase matched mixture of xenon and argon. At other wavelengths the output of a Nd:YAG pumped dye laser is mixed with residual 1064 nm radiation in a KD*P crystal to produce tunable UV radiation from 360 to 390 nm. This radiation is then focused in a mixture of either xenon or krypton phase matched with argon.

RESULTS: The argon is used to compensate for the negative dispersion of the krypton or xenon. For third harmonic generation to occur under focused conditions the generating medium must be negatively dispersive. Regions of negative dispersion occur to the high energy side of atomic transitions. The amount of negative dispersion can be optimized either by adjusting the rare gas pressure or by compensating with a second, positively dispersive, gas. By using a second gas it is possible to increase the partial pressure, and the efficiency, of the third harmonic medium while maintaining the optimum dispersion conditions.

Although the efficiency of third harmonic generation is increased by using a positively dispersive gas to optimize the dispersion, the wavelength region of efficient conversion is substantially decreased. This is shown below in the wavelength scans for pure Kr (10 torr) and for a mixture of 25% Kr in Ar at 350 torr. Because coherent VUV generation is usually used for scanning experiments the narrow wavelength region of the mixture is unacceptable. However, for VUV photoionization experiments it is not necessary to scan wavelength so it is possible to take advantage of this increase in efficiency.

Improved Efficiency With Phase Matched Mixtures:

Optimized Coherent VUV Wavelengths and Efficiencies:

<table>
<thead>
<tr>
<th>VUV (nm)</th>
<th>THG Gas</th>
<th>% Ar in Mixture</th>
<th>Relative VUV Output</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pure Mix</td>
<td>Pure</td>
<td>Mixture</td>
</tr>
<tr>
<td>118.2</td>
<td>Xe</td>
<td>8.3%</td>
<td>5.7</td>
</tr>
<tr>
<td>121.5</td>
<td>Kr</td>
<td>28.0%</td>
<td>1.0</td>
</tr>
<tr>
<td>122.8</td>
<td>Kr</td>
<td>8.0%</td>
<td>1.1</td>
</tr>
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<td>122.9</td>
<td>Kr</td>
<td>6.0%</td>
<td>0.4</td>
</tr>
<tr>
<td>123.2</td>
<td>Kr</td>
<td>4.0%</td>
<td>0.7</td>
</tr>
<tr>
<td>127.0</td>
<td>Xe</td>
<td>50.0%</td>
<td>7.9</td>
</tr>
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<td>Xe</td>
<td>25.0%</td>
<td>6.1</td>
</tr>
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<td>128.2</td>
<td>Xe</td>
<td>12.0%</td>
<td>3.1</td>
</tr>
<tr>
<td>128.9</td>
<td>Xe</td>
<td>4.0%</td>
<td>0.6</td>
</tr>
</tbody>
</table>

318
An interesting trend is seen in the Table above. As the wavelength increases the optimum mixing ratio decreases. This can be understood in terms of the negative dispersion per atom, which increases as the atomic transition (123.58 nm for Kr and 129.6 nm for Xe) is approached. As a result, a higher relative amount of argon is required to compensate for the increasing negative dispersion. By adjusting the relative amount of Ar it is possible to select a rare gas mixture which is most efficient at a selected VUV photoionization wavelength.

SOFT IONIZATION: The ability to select a photoionization wavelength makes it possible to control fragmentation. The wavelength dependence of fragmentation was studied for a number of functional groups which typically show little or no molecular ion. \( n \)-Alkanes, alkenes, ketones, carboxylic acids, ethers, amines, and aldehydes all form predominant molecular ions at low photoionization energies. The results for octanal, which fragments extensively above 9.6 eV, are shown below. A number of interesting fragments are seen at higher energies, including the metastable loss of \( \text{H}_2\text{O} \) (m/z 110), the McLafferty ion (m/z 84), and two \([\text{M-}2\text{nC}_{2n}\text{H}_{4n}]^+\) (m/z 72 and 100) fragments. As the photoionization energy is reduced the relative abundance of these fragments changes, indicating the K vs E dependence for each fragment. Finally, a change of only 0.7 eV eliminates almost all fragmentation. This strong wavelength dependence of fragmentation indicates that Tunable VUV photoionization will be very useful in experiments which require soft, universal ionization.

Octanal Photoionization

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<tr>
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</table>

\( m/z \)
SINGLE PHOTON IONIZATION OF NEUTRALS PRODUCED BY ULTRAVIOLET LASER DESORPTION

C.D. Mowry and M.V. Johnston, University of Delaware, Department of Chemistry and Biochemistry, Newark, DE 19716

INTRODUCTION: A linear time-of-flight mass spectrometer allowing photoionization of ultraviolet laser desorbed neutrals is described. Single photon ionization is achieved using coherent vacuum ultraviolet radiation at 118 nm. Laser desorption (LD) and photoionization (PI) are both performed in the source region of the mass spectrometer. LD/PI provides qualitative information on the internal and kinetic energies of laser desorbed neutral molecules. Initial results involving nicotinic acid and a nicotinic acid:octyl amine solution are presented.

EXPERIMENTAL: An excimer laser (248 nm) is used for desorption. The laser beam is focused to 150 x 100 μm on the desorption probe at an angle 48° from the flight tube axis. Samples are deposited in μl quantities and air dried. The probe can be rotated 360° to access fresh sample surface, and vacuum is maintained during probe removal and insertion. Vacuum ultraviolet (VUV) radiation generated by frequency tripling the third harmonic of a Nd:YAG laser is used to ionize neutral desorption products. An off-axis optical alignment allows the 118 nm radiation to be focused a few mm from the probe surface along the flight tube axis, whereas residual 355 nm radiation is directed several mm below the plane of the flight tube. Primary desorption ions are rejected using a pulsed voltage applied to the backing plate of the source region. A delay generator controls the timing of the voltage pulse and also the delay between LD and PI lasers. The MCP detector signal is collected using a digital oscilloscope.

RESULTS: The LD spectrum of nicotinic acid in Figure 1 shows the molecular and fragment ions produced. LD/PI also produces molecular and fragment ions for nicotinic acid. The presence of a metastable tail in LD/PI for m/z 105 suggests this ion is produced by unimolecular dissociation of M* after photoionization. The gas phase mass spectrum of nicotinic acid does not contain any fragment ions, indicating that 10.5 eV alone is not energetic enough to cause fragmentation at 100°C. These observations imply that the desorbed neutrals have high internal energy.

LD and LD/PI spectra of a 5:1 molar solution of nicotinic acid:octyl amine is shown in Figure 2. Laser desorption of this sample shows only MH* for octyl amine and no fragment ions attributable to octyl amine. A fragment ion at m/z 30 is the only predominant ion observed from octyl amine in the LD/PI spectrum. The spectrum is similar to 70 eV mass spectra of octyl amine. Gas phase laser desorption products of octyl amine at room temperature creates metastable fragments but also an abundant parent ion. Laser desorbed octyl amine neutrals therefore also have a high internal energy.

Ion peak widths in LD/PI mass spectra are as narrow as those of gas phase spectra obtained in this instrument. This indicates a small distribution of kinetic energies for the desorbed neutrals. Broader peaks would be expected in LD/PI if the kinetic energy distribution was large. The small kinetic energy distribution in LD/PI suggests two possibilities: 1) the kinetic energy distribution of desorbed neutrals is different than that of desorbed ions 2) the broadening of peaks in LD is due to some factor other than kinetic energy.

LD/PI should be useful for gaining information about mechanisms, characteristics, and energetics of laser desorption. The capacity exists currently for tunable VUV radiation from 9.6 to 10.5 eV, which should permit selective ionization of desorbed neutrals. The variable delay between LD and PI events will allow investigation into the kinetic energy distribution of desorbed neutrals under various conditions.
Aryltriphénylphosphonium Halides as "Molecular Thermometers" in Matrix Assisted Laser Desorption

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Matrix assisted laser desorption (MALD) exhibits unique capabilities in the volatilization of large organic molecules. Proteins with molecular masses exceeding 200 kDa were transferred intact into the gas phase utilizing this method [1]. Our aim was to investigate the mechanism of volatilization with special emphasis on the temperatures experienced by the guest molecules embedded in the matrix.

Triphenylphosphonium salts are known as thermally labile molecules. Moreover, their fragmentation pathways (simple cleavage versus rearrangement) reveal their internal energy distributions [2,3]. Therefore, we used them as "molecular thermometers" to probe both the temperature experienced by the guest molecules and their internal energies.

Laser desorption mass spectra of four different phosphonium salts in neat form and in nicotinic acid matrix (one of the best matrices in MALD) were measured using frequency quadrupled Nd-YAG laser for excitation. Glycerol and ammonium chloride matrices were also tried with benzyltriphenylphosphonium chloride (Table 1). All four phosphonium salts exhibit three characteristic reaction channels: intact cation formation, thermal decomposition and major fragmentation of the intact cation. These three possibilities are demonstrated for 1-naphthylmethyl-triphenylphosphonium chloride in Figure 1. The mentioned pathways have been studied and confirmed separately by electron impact, fast atom bombardment and laser desorption mass spectrometry [2,3].

The intensity ratio of thermal decomposition products to intact cation, \( \frac{I(m/z=401)+I(m/z=402)}{I(m/z=403)} \), is considered to be a direct measure of the thermal load on the phosphonium salt during the laser desorption process. The ratio of the fragmented cation intensity to the intact cation intensity, \( \frac{I(m/z=141)}{I(m/z=403)} \), is thought to be related to the internal energy of the desorbed cation. The change of these ratios as a consequence of nicotinic acid matrix addition to 1-naphthylmethyl-triphenylphosphonium chloride is displayed in Figure 2.

UV laser desorption time of flight mass spectra of the other neat aryl-triphenylphosphonium chlorides and the corresponding matrix assisted spectra also revealed dramatic differences in thermal processes and in fragmentation patterns. The lack of thermal degradation and clear indications of significantly lower internal energies were observed in the matrix assisted case in accordance with the predictions of our cool plume [4] and homogeneous bottleneck model [5].

Similar measurements with glycerol and ammonium chloride matrices showed similar, though greatly reduced "cooling" effect of these substances. Their reduced efficiency is also feasible in the framework of the cool plume model [4] with respect to the higher volatilization temperature and/or to the lower UV absorption of these materials.

Table 1. Different phosphonium salts used as "molecular thermometers" and the selection of matrices.

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<td>nicotinic acid</td>
</tr>
<tr>
<td>4-(4'-methylystyryl)benzyl-triphenylphosphonium chloride</td>
<td>glycerol</td>
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<tr>
<td>1,4-di(triphenylphosphoniomethyl)benzene dichloride</td>
<td>ammonium chloride</td>
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Figure 1. Ionization pathways of 1-naphthylmethyl-triphenylphosphonium chloride through thermal decomposition ($\Delta T$) and in the absence of thermal decomposition (no $\Delta T$).

Figure 2. Laser desorption mass spectra of neat 1-naphthylmethyl-triphenylphosphonium chloride (upper) and of the same compound in nicotinic acid matrix (lower). Thermal decomposition is apparent in the case of the neat sample (upper right inset) whereas no thermal decomposition is present in the matrix assisted case (lower right inset). The left insets are showing the suppression of cation fragmentation.

**K+IDS ANALYSIS OF SEVERAL ORGANIC SALTS AND A COMPARISON OF THE RESULTS TO OTHER DESORPTION/IONIZATION METHODS**

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The desorption/ionization method used here, potassium ionization of desorbed species (K+IDS), has worked well for a number of compound classes. The experimental procedure is simple and requires no modification to the mass spectrometer. A number of desorption/ionization (DI) methods have been used to analyze organic salts. We have chosen to target select penicillins and dyes due to their thermal instability and interest within the mass spectrometry community. By comparing these results to those obtained using other DI methods we can assess the utility of the K+IDS method in analyzing these compounds.

K+IDS is a technique that can be used to analyze thermally labile compounds at a much lower cost than alternative DI methods. K+IDS can be used with any mass spectrometer fitted with a direct insertion probe inlet. The probe used in this method is inexpensive and simple in construction. A solution containing the analyte is deposited on the probe and allowed to dry. The probe tip consists of a ceramic doped with an alkali salt (in this case potassium sulfate). Once inside the ion volume of the mass spectrometer the probe is rapidly heated to a temperature sufficient to emit potassium ions from the alkali ceramic.

The mechanism of K+IDS involves the interaction between potassium ions (thermionic emission) and desorbed species arising from the surface of the thermionic material. The desorbed species are either intact molecules and/or thermal decomposition products of the analyte. Under certain conditions the desorbed species may leave the surface as ions (i.e., surface ionization). The distribution of these products depend upon the heating rate of the alkali ceramic. Simply, K+IDS is a potassium ion chemical ionization method coupled with rapid heating.

Figure 1 shows the K+IDS spectrum of penicillin G procaine salt. This spectrum contains ample structural information and the molecular weight of the salt. (The ion at m/z 609 is the potassiated adduct of the intact salt). In cases where the cation is an alkali metal (e.g., penicillin G potassium salt) poor spectra are obtained. By adding ammonium chloride to the sample prior to deposition on the K+IDS probe, the spectra begin to resemble the results obtained from analytes which contain an organic cation.

Figure 2 shows a K+IDS spectrum of methylene blue obtained using a rapid heating rate. The most abundant ion (m/z 284) is the intact cation. The other ions present are believed to be intact cations of impurities present in the sample (e.g., m/z 270 and 256). This is not surprising from literature accounts of the types of impurities found in this dye.

For this spectrum the observed ion intensities parallel the reported purity for this sample (85%). As with other K+IDS analyses performed in our laboratory, quantitation of a mixture is best done using a rapid heating rate as possible. This minimizes the probability of decomposition products being formed which would complicate quantitation.

The use of an alkali ceramic material for the emission of potassium ions creates the possibility of utilizing the K+IDS method for the off-line analysis of thin layer chromatograms. Methylene blue was chosen to demonstrate this approach since it was known to contain two other contaminant cations at m/z 256 and 270. These cations are structurally the same except with differing methyl constituents. Thin layer chromatography on a 250 ng spot of methylene blue sample produced three well-defined spots. The silica containing each analyte was collected from the plate and a slurry was made which was deposited directly onto the thermionic probe tip. The silica containing the analyte proved to adhere well to the alkali ceramic material of the probe tip. The results of the three analyses are listed in the following table.

The most abundant ion in each spot is the cation for that analyte. Small amounts of the other cations are observed in each spectra due to poor chromatography and/or memory effects. It is clear that the use of the K+IDS technique is applicable to thin layer chromatographic analysis and has certain advantages over other DI methods which have been used in a similar fashion. In DI methods using a beam (e.g., SIMS), ions are only generated when analyte is exposed to the beam.
The amount of analyte exposed may be only a small fraction of the total sample. If this is the case, sensitivity may be a problem. In addition, K+IDS is a technique which can be rapidly performed therefore reducing analysis time.

In summary, this technique provides similar information as many other DI methods. The major advantages of K+IDS over other methods are cost savings and simplicity. The results presented here suggest the following conclusions.

1. Spectra are similar to other DI methods and are influenced by the heating rate employed.
2. Appropriate additives to the sample can influence spectral features.
3. Salts of organic dyes generate spectra lacking alkali ion adducts. Although this is the case a fast heating rate is still required.
4. Molecular weight information and structural information can be generated in this approach.
5. The use of the K+IDS technique for off-line TLC analysis is useful and provides advantages over other DI methods.

FIGURE 1

FIGURE 2

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<th>m/z 284</th>
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<tr>
<td>SPOT #3</td>
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ON-LINE SINGLE PARTICLE ANALYSIS
BY LASER DESORPTION MASS SPECTROMETRY

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Introduction  Mass spectra of both organic and inorganic particles in the 3-10 \( \mu \)m range have been obtained by single-step, laser desorption/ionization. This method offers a few distinct advantages over other current methods. Complete mass spectra are obtained for each particle in an on-line mode. Also, sample integrity is maintained in two important ways. First, sample contamination and/or decomposition is reduced since particle/surface interactions are virtually eliminated. Second, the loss of volatile components is reduced since the particles spend less than a millisecond under vacuum conditions before ionization.

Experimental  Particles of known size and composition were generated and directed into the source region of a linear time-of-flight mass spectrometer through a differentially pumped nozzle assembly. The system is outlined in figure 1.

Particle detection in the source region of the mass spectrometer was accomplished by monitoring scattered radiation from a He-Ne laser. The He-Ne crossed the source region perpendicular to the flight region and was focused to a 100 \( \mu \)m spot. The scatter was collected by a fiber optic, detected by a photomultiplier, amplified and sent to a discriminator. The output of the discriminator was used to trigger an excimer laser for particle desorption/ionization. Ions were detected using a microchannel plate detector. The output was digitized with an oscilloscope or first logarithmically amplified.

Results  Figure two shows a mass spectrum of one 9 \( \mu \)m mixed salt particle. The particle was composed of NaCl, KCl and (NH₄)₂SO₄. The initial salt solution was prepared in a 4 : 1.5 : 2 molarity ratio, respectively. The laser was operated at 193 nm and at a pulse energy 50 MW/cm². The spectrum is dominated by K⁺, Na⁺, and NH₄⁺ yet contains a large number of cluster ions similar to those seen in LAMMA experiments. The largest peak in the spectrum is K⁺, however KCl in the salt solution was the least concentrated. This result may be due to the relatively low ionization potential for potassium or the result of particle concentrations differing from that of the original salt solution. The threshold for particle desorption was found to be approximately 25 MW/cm². Mixed salt particles of 6 and 9.5 \( \mu \)m were studied under the same experimental conditions. This difference represents a factor of 4 change in mass. However no significant change in ion current was observed, indicating incomplete desorption of the particle. Particles of less than 3 \( \mu \)m diameter could not be accommodated with the current optical configuration due to the lack of a baffle system preventing stray light from reaching the photomultiplier.

Figure three demonstrates the potential application of the logarithmic amplifier. The top spectrum is an expanded region of a salt particle spectrum. Weak features in the spectrum can be lost due to the limited dynamic range. The second spectrum was obtained from the same particle. The ion current was logarithmically amplified and then converted back to a linear form. The extended dynamic range is clearly demonstrated.

Two ion trajectory programs, SIMION and SIMTOF, were used to model single particle laser desorption. Two source region configurations were used for experimental and modelling work. The drawout voltage was varied from 2300 V to 0 V. Particle to particle variations in flight times for both configurations could be explained and predicted based upon the position of the particle in the excimer beam upon desorption. The peak widths were explained using an ion formation region slightly larger than the particle (10-20 \( \mu \)m) and an ion temperature between 1,000 and 10,000 K.
Figure 1

Figure 2

Figure 3
Lucinda Kittle, Andrew G. Sharkey, Badie Morsi and David M. Hercules
Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260

Reduction of pyritic sulfur and mineral matter in coal is important in controlling emission of sulfur gases and particulate matter during coal combustion. Coal cleaning techniques such as agglomeration are being investigated to selectively remove pyrite and mineral matter while maintaining the original BTU content of the coal. Following changes in the surface composition of the coal with treatment is important for understanding the mechanisms of the cleaning techniques. Proper control of the coal surface properties will improve the effectiveness of agglomeration in producing premium quality clean coal. Our studies involved monitoring changes in the near surface elemental composition with various agglomeration procedures, and correlating these changes with those observed by bulk techniques.

The Laser Microprobe Mass Analyzer has previously been used as a tool for mapping coal macerals. Changes in elemental surface composition can also be determined. Coal from Illinois #6, Pittsburgh #8, and Upper Freeport #3 seams was analyzed using the Leybold Heraeus LAMMA-1000, which has been described elsewhere. Samples were prepared by mounting the coal powder on double-sided sticky tape supported by zinc foil. The 250x microscope was used to select areas on the surface of individual coal particles. Fifty spectra were collected and averaged from each individual particle. A total of 10 particles were analyzed from each sample. This procedure was developed in order to compensate for the inhomogeneity of the coal surface and the variations in instrumental factors. The relative amounts of the following elements were semi-quantitatively determined: Li, Na, Mg, Al, Si, S, K, Ca, Ti, and Fe. The results were presented in terms of percent total ionization for the 10 elements. While the percent total ionization does not represent absolute concentrations, trends in concentration following physical and chemical treatment can be followed. Changes in the percent total ionization will be proportional to changes in the concentrations of the elements.

Coal cleaning through agglomeration consists of a preliminary grinding step during which the coal is ground in water with additives. Results of agglomeration of Pittsburgh #8 wet ground without additives were obtained in order to establish a base set of data. Feed, product and refuse samples from agglomeration were examined to determine the elemental composition. After successful agglomeration cleaning the concentration of pyrite should increase in the refuse. The elemental distributions for the 3 fractions from agglomeration for wet ground Pittsburgh #8 coal are shown in Figure 1. These samples showed for iron and sulfur a decrease in the percent total ionization from the feed to product and an increase from feed to refuse. Mg, Li, and Si also increased in the refuse. Na, Al, Ca, and Ti increased in concentration in the product and decreased in the refuse.

The addition of tall oil during grinding was studied in order to determine its effect on the elemental distributions of the 3 agglomeration fractions. Pittsburgh #8 coal wet ground with 200 ppm tall oil added showed similar behavior compared to the coal wet ground without additives. The iron and sulfur decreased in % total ionization in the product, and increased in the refuse. The addition of tall oil had no significant effect on the elemental composition of Pittsburgh #8 coal.
Agglomeration treatments were conducted using various hydrocarbon to coal ratios. It was postulated that addition of hydrocarbons during grinding will aid in the removal of pyrite. Illinois #6 wet ground base coal with no additives during grinding was first analyzed by LAMMA. Illinois #6 coal with a pentane to coal ratio of 1:5 was then analyzed to determine the effect of grinding additives on the surface elemental composition. The results for the % total ionization of the 10 elements for these 2 samples are shown in Figure 2. The addition of pentane had little effect on the elemental composition, with most elements showing little or no change in % total ionization. The iron and sulfur showed small increases in % total ionization for the pentane ground coal compared to the wet ground coal without additives. Na and K showed small decreases in % total ionization, which leads to speculation that the removal of these highly soluble cations from the surface of mineral matter may expose more pyrite resulting in increases in sulfur and iron surface concentration.

LAMMA analysis can be a useful technique in evaluating the success of physical and chemical treatments of coal. These results have been successfully correlated with bulk analysis data.

Acknowledgment: This work was supported by the Department of Energy Contract No. DE-AC22-88PC88877.

References
NECESSITY OF JET-COOling OF LASER-DESORBED MOLECULES IN THE MPI-ION-SOURCE OF A TOF MASS SPECTROMETER

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Laser desorption of neutral molecules followed by supersonic jet cooling and multiphoton ionization has become a well-known technique to examine labile, non-volatile molecules; many results have been published (see references in [1]). An interesting alternative to this ion source for a time-of-flight mass spectrometer is the laser desorption of neutrals with subsequent multiphoton ionization, located as close as possible to the place of desorption (i.e. without using a jet) [2]. The latter technique should at least be more sensitive, but up to now, results are reported only for small or stable compounds.

The Bruker TOF-1 time-of-flight mass spectrometer has been used to perform comparative measurements of laser desorption of neutrals without (ND) and with (ND-jet) subsequent cooling by a supersonic jet followed by resonance-enhanced multiphoton ionization (MPI). Neutral species were desorbed by means of a CO2 laser pulse (10.6 μm, 10 μs) and postionized by a UV laser pulse (270 nm, 8 ns). The ions are accelerated into a TOF mass spectrometer and detected with a dual microchannelplate detector.

The measurements have been performed for a series of 5 peptides with increasing number of amino acids labeled below as P1-P5: Tryptophan, GLY-PHE, GLY-LEU-TYR, Leucine-Enkephaline and Pentagastrin. Figure 1 gives as an example the results for Pentagastrin. (a) gives the spectrum for ND-MPI (b) gives the spectrum for ND-jet-MPI and (c) for ND-jet-EI i.e. electron impact ionization (EI) is used instead of multiphoton ionization (MPI).

For ND-jet-MPI the molecular ion is clearly observed for P1-P5 as a peak which has about the highest intensity. The power of the IR-laser was kept for all spectra at a constant high value of about 10^9 W/cm². The intensity of the ionization laser was also kept at a constant medium value of 10^7 W/cm² to produce not only molecular ions, but also some significant fragments. As wavelength 270 nm was chosen. For ND-MPI, the molecular ions are observed only for P1-P3. For P4, higher mass fragments can be seen (432 u and 449 u), and for P5, only small fragments are observed. In each case, especially for P4 and P5, we tried very carefully to obtain as much molecular ion signal as possible. The power of the desorption laser was reduced to the desorption threshold. When proceeding to higher mass peptides, we observed that the threshold power was increasing, i.e. more energy is needed for P5 to be desorbed than for P1. For ND-jet-EI, similar results as for ND-jet-MPI can be obtained, as shown in Fig. 1c. For more results with ND-jet-EI see [3].

Thus, the jet cooling is inherently needed, if peptides containing 4 and more amino acids are to be examined using neutral desorption (10 μs IR-pulses) and postionization. As can be seen from the EI experiments, the jet cooling is the important step, because even with the hard EI-ionization molecular ions are observed for P5. We conclude that the neutrals taking up some amounts of initial energy during desorption are able to survive as intact molecules as long as they are neutral. But after ionization they im-
mediately fragment due to the lower fragmentation thresholds of the ions. If, however, the internal temperatures of the molecule are cooled down after desorption (and before ionization) they have a chance to survive the ionization process as intact molecules no matter whether they are ionized by MPI or by EI. With the same argumentation an effect so far not understood can be explained: With ND-jet-MPI, molecular ions of labile samples with masses above 3000 are not observed as yet. Those high masses are heated even higher in the desorption step and the jet is no longer able to cool them to sufficiently low temperatures. Thus, they are fragmentized after ionization for the same reasons as lower mass peptides when no jet cooling is applied.


![Pentagastrin Mass Spectra](image)

Fig. 1: Mass spectra of Pentagastrin. (a) Neutral desorption followed by immediate multiphoton ionization, (b) Neutral desorption - jet cooling - multiphoton ionization, (c) Neutral desorption - jet cooling - electron impact ionization
Introduction
Matrix-assisted laser desorption time of flight mass spectrometry (LDTOFMS) has rapidly become one of the forefront techniques for the mass spectrometric analysis of large biomolecules. Alterations of the original technique /1/ by Beavis and Chait has enabled the highly accurate molecular weight determinations of large proteins while maintaining pmol to sub-pmol sensitivity /2/. Added benefits of the Beavis and Chait technique are an immunity to large concentrations of contaminants, selective ionization of protein analytes and the ability to readily analyze complex mixtures. These benefits make LDTOFMS the mass spectrometric technique of choice for the direct analysis of naturally occurring protein mixtures, as demonstrated on the analysis of human and bovine milk /3/. We have analyzed several protein mixtures obtained directly from the biological source, including human milk whey and direct extracts of plant membrane proteins /16/. This communication presents the direct analysis, and partial identification, of human salivary proteins.

Experimental
Approximately 1 ml of human saliva was obtained and diluted by a factor of 10 with 0.1% aqueous TFA. To 1 microliter of the diluted saliva, 1 microliter of matrix solution (saturated sinapinic acid in 30:70, acetonitrile:0.1% aqueous TFA) was added. The combined 2 microliters was allowed to air dry on an insertion probe. Mass spectrometry was performed on a Vestec Model 2000 laser desorption time of flight mass spectrometer operating at 30 kV accelerating potential. The mass spectrum shown was obtained through the signal averaging of 100 laser shots (Nd:YAG laser operating at 355 nm). To obtain reasonable ion signal throughout the entire mass region investigated, a variation in laser power density at the sample was needed, i.e. a higher laser power density for the mass range > 16,000 Da. Two factors could contribute to this: a) the component analytes being in solution in varying concentrations, and, b) a lower detection efficiency for the higher mass ions. Unfortunately, when going to a higher laser power density to observe the larger ions, resolution of the lower mass ions was sacrificed. Mass calibration was performed prior to and following the analysis of saliva using apomyoglobin (horse heart MW = 16,950.7 Da). Molecular weight determination error, using an external calibrant, was estimated to be ±0.05%. Molecular weights of salivary proteins were calculated from established sequences using PROCOMP /4/.

Fig. 1 Matrix-assisted laser desorption time of flight mass spectrum of human saliva.
Results and Discussion

Fig. 1 shows the mass spectrum obtained from human saliva. Over 30 of the ion peaks were determined to represent component protein analytes i.e. their m/z values could not be justified by multiply charged values of other peaks or values derived from matrix adducts. Table I shows 14 salivary proteins identified using molecular weights calculated from known sequences. All assignments agree within 0.1% of the calculated masses with the exceptions of Protein C and amylase (for which no sequence data could be found). Molecular weights for salivary proteins PRP-IB6 and PRP-IB9 were calculated from their known sequences /15/, yet neither were observed in this saliva sample. In that only ~17% of the population secrete these proteins /15/ this is not surprising, however, PRP-IB6 has been observed in saliva samples obtained from other subjects /16/.

<table>
<thead>
<tr>
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<td>Protein C</td>
<td>15,510.4</td>
<td>15,529.0</td>
<td>13</td>
</tr>
<tr>
<td>Amylase</td>
<td>56,000**</td>
<td>56,250</td>
<td>14</td>
</tr>
</tbody>
</table>

* The m/z value identified as PRP-H could also be Statherin+matrix or Protein A doubly charged.
** Estimated from SDS PAGE.

Conclusion

Demonstrated is the ability of matrix-assisted laser desorption time of flight mass spectrometry to yield useful molecular weight data on complex naturally occurring protein mixtures, with no prior separation. This, coupled with the inherent sensitivity of the technique, suggest the possible use of LDTOFMS as a means of monitoring bodily proteins at quantity levels which are not taxing to the subject.

References

4. PROCOMP Andrews P.C., University of Michigan.
Sample preparation in desorption ionization techniques such as FAB, SIMS, PDMS, etc. employ the use of various matrices that play a role in the desorption and ionization of analyte molecules. Similar to other desorption techniques the matrix plays a key role in matrix assisted laser desorption (MALD). The interactions between matrix and analyte can be understood by considering our current view of the MALD process. The steps include: 1) photon absorption to produce an ensemble of excited state matrix molecules (S); 2) internal conversion of some S, species results in heating of a localized region; 3) the rapid heating causes ejection of "cluster species" from the surface, e.g. [(S)n—S] or [(S)n(A)m—S], where A represents analyte molecules; 4) the enhanced acidity of S, (relative to S0) results in proton transfer to yield [(S)n(A)m—AH+—S] or [(S)n—S] or intact analyte molecular ions [A+H]+.

The temporal profiles of mass selected ions produced by MALD is a direct measure of the time for formation of ions plus the resident time of the ions in the source. The time requirement for ion formation is governed by the mechanism of the ionization process. Figure 1 shows the temporal profile for the [M+H]+ ion of sinapic acid. The peak is bimodal composed of a sharp peak and a tailing distribution that extends beyond 50 usec. The ratios of ions in the two portions of the profile (short time for arrival vs. long time for arrival) varies from approximately 1:1 to 1:10. Although we have not made measurements of the KE distribution of the two components contained in the profile, preliminary measurements show that component A ions have higher kinetic energies than...
component B ions. Our interpretation of the arrival time distribution corresponds to ions formed directly (either [M+H]+ or small clusters) where the time scale for evaporation is short with respect to the ion source residence time (approximately 1 usec). Component B, on the other hand, arises from the ejection of "large" cluster species. These large cluster species also evaporate, but the time required for evaporation is longer because the number of solvent molecules is "large". We are now performing experiments using accelerating voltage metastable ion scans in an effort to determine the distribution of cluster size. For example, accelerating voltage metastable ion scans will allow us to identify the parent ion(s) of a particular daughter ion;

\[ [M+H]^+ S, \rightarrow [M+H]^+ S_{n+1} + aS. \]

The overall shape of the temporal profile is dependent upon a number of experimental parameters, e.g., laser power density, ion source focussing "ion draw-out potential", matrix to analyte ratio, liquid matrix vs solid matrix, etc., but under these circumstances we continue to observe a bimodal peak that contains components A & B.

\[\text{FIGURE 1}\]

\[\text{Counts}\]

\[\text{Time (ps)}\]

\[^{1}\text{Cheshnovsky, O.; Leutwyler, S.: Chem. Phys. Lett. 1985,121, 1.}\]
Sample preparation in desorption ionization techniques such as FAB, SIMS, PDMS, etc. employ the use of various matrices that play a role in the desorption and ionization of analyte molecules. Similar to other desorption techniques the matrix plays a key role in matrix assisted laser desorption (MALD). The interactions between matrix and analyte can be understood by considering our current view of the MALD process. The steps include: 1) photon absorption to produce an ensemble of excited state matrix molecules \((S)\); 2) internal conversion of some \(S\) species results in heating of a localized region; 3) the rapid heating causes ejection of "cluster species" from the surface, e.g. \([(S)(n)\rightarrow S]\) or \([(S)(n)\rightarrow (A)(n)\rightarrow S]\), where \(A\) represents analyte molecules; 4) the enhanced acidity of \(S\) (relative to \(S\)) results in proton transfer to yield \([(S)(n)\rightarrow (A)(m)\rightarrow AH^+\rightarrow S]\); 5) evaporation of the cluster yields solvated adducts, \([(S)(n)\rightarrow AH^+]\) and \([(S)(n)\rightarrow S]\) or intact analyte molecular ions \([A+H]^+\).

The key component of the matrix assisted laser desorption mechanism that we have proposed is the enhanced acidity of the absorbing matrix in the excited state. With this in mind consider the resonance structures of sinapic acid shown in figure 1. Upon photon absorption the acidity of the phenolic proton is believed to be increased by delocalization of the lone pair of electrons on the adjacent oxygen through resonance effects. Organic solute interactions that shift the equilibrium toward the right (figure 1), may be useful in enhancing the population of acidic hydrogens available for proton transfer in the excited state. The hydrogen bonding interactions shown in figure 2 may shift the equilibrium of the species represented in figure 1 by stabilizing the charged...
carboxylate group of the Sinapic acid.

FIGURE 1

FIGURE 2

LIGAND-PROTEIN AND PROTEIN-PROTEIN BINDING STUDIES
WITH MATRIX-ASSISTED LDMS.
*Marc J.-F. Suter, William T. Moore, John S. Cottrell and Richard M. Caprioli
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INTRODUCTION
Since its introduction in 1987, matrix-assisted laser desorption mass spectrometry (LD-MS) has
become increasingly popular in the field of biochemistry. A wide variety of matrices and wavelengths have
been investigated with the intention of reducing the matrix induced chemical noise and increasing the
applicability of the method to different classes of molecules. The most widely used matrices to date are
nicotinic acid (266 nm, Nd-YAG laser) and sinapinic acid (266 nm, dye laser; 337 nm, N₂-laser; 355 nm Nd-
YAG laser) and its homologues. Time-of-flight mass spectrometers are easy to build and the focus has
been on N₂-lasers, which are inexpensive and thus reduce the overall cost of an instrument.

Of interest to biochemists is the composition of multimers, such as enzymes, verification of
hybridization, identification of ligands and detection of chemical modifications. Even though LD ionization
is a so called soft technique, only very few examples of enzymes surviving the ionization process as intact
multimers (e.g. streptavidin) have been reported. Karas et al. showed the influence of the alcohol content
on the dissociation of multimers into subunits. Nicotinic acid is a water soluble compound, which allows
for a less denaturing environment, whereas sinapinic acid is not soluble in pure water. This is possibly
the reason why no multimers have been reported so far in conjunction with the sinapinic acid matrix.

PROTEINS INVESTIGATED
Alcohol dehydrogenases (ADH) catalyze the oxidation of alcohols. Yeast ADH exists as two
isozymes that differ in 23 amino acids. Each isozyme is reported to consist of 4 identical subunits. Horse
liver ADH is a dimer of two different subunits. Comparing the two enzymes shows an inhomologous primary
structure. Even so, the tertiary structures are very similar and all main enzymatic mechanisms are common
to both proteins. On the other hand, the structural deviations affect the subunit interaction and it is
expected that the quaternary structure of the two proteins will be different.

Avidin is a glycoprotein composed of 4 essentially identical polypeptide chains. It forms a very
strong complex with d-biotin, a fact that has been widely exploited for the design of affinity columns. The
binding constant is in the order of 10⁻¹⁵ M and the biotin binding ability occurs between pH values of 2 to
13, and at guanidine hydrochloride concentrations of up to 3M, reflecting the stability of the complex.
Binding of biotin by single subunits was observed with a binding constant of 10⁻⁷ M.

RESULTS AND DISCUSSION
The two forms of ADH investigated have very similar tertiary structure with the exception mentioned
above, but are expected to differ in their quaternary geometry. Indeed y-ADH shows an increased signal
for the dimer, whereas with h-ADH, only the monomer can be detected.

The average molecular weight thus determined for y-ADH is 36'915 (σ₁₈ = 37) for the monomer and
73'540 (σ₁₈ = 195) for the dimer. These values are high by 140 - 160 u for the monomer but well within
the margin of error for the dimer. The deviation for the monomer possibly reflects the presence of zinc
ions.

Verification of the dimeric form of y-ADH was done with gel filtration. Treatment of y-ADH with
denaturing agents (3M guanidine hydrochloride or NH₄HCO₃) dissociates the dimer predominantly into
monomeric subunits, thus proving that the dimeric form seen with LD-MS is not covalently linked.
Electrospray spectra of untreated y-ADH support this finding.

The avidin/biotin system was chosen as a model compound for the investigation of ligand-protein
binding, because it has a very strong affinity for biotin, even under what is normally considered denaturing
conditions. However, no evidence of multimers and biotin binding was detected.

EXPERIMENTAL CONDITIONS
The laser desorption time-of-flight mass spectrometer used has a 50 cm flight tube and is operated
at an accelerating voltage of 20kV. A N₂-laser yields an adjustable power density of around 10⁶ W/cm².
Data acquisition is done with a LeCroy 9450 digital oscilloscope. ADH was dissolved in water at a concentration of approx. 10 pmol/μl, sinapinic acid in 50% EtOH or 70% ACN.

Gel filtration was performed on a Sephadex G25 column (1.4x29.0 cm) at a flow rate of 0.9 ml/min with 1% NH₄HCO₃ at pH 8. The sample concentrations were 10 pmol/μl, 700 μl were loaded. y-ADH was denatured in 3M guanidine hydrochloride.

CONCLUSION

The sinapinic acid/337 nm combination can be used to determine multimeric protein structures under certain conditions, although it appears to be less generally applicable relative to the nicotinic acid/266 nm experiment.

In the case of y-ADH, the hydrophobic forces holding the subunits together were sufficiently strong to prevent dissociation under the conditions used for sample preparation. This was not the case for avidin/biotin binding, which showed only monomeric avidin on LD-MS analysis.

Current and future studies are aimed at elucidating the critical parameters of the y-ADH system to better understand conditions necessary for the observation of multimeric protein complexes.

ACKNOWLEDGMENT

This work was supported by a grant from the National Institute of Health (grant # GM43783-02).

Two-Step Laser Desorption / Multiphoton Ionization Mass Spectrometry: Applications and Mechanisms

C. R. Maechling, R. Zenobi, L. J. Kovalenko, S. J. Clemett, R. N. Zare
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The capacity of laser desorption to volatilize intact neutral molecules combined with the high sensitivity and molecular selectivity of resonance-enhanced multiphoton ionization (REMPI) make two-step laser mass spectrometry (L\textsuperscript{2}MS) a powerful technique for the organic analysis of unique cosmochemical and geochemical samples. The utility of L\textsuperscript{2}MS has been demonstrated by the in situ analysis of polyaromatic hydrocarbons (PAHs) in both carbonaceous [1] and ordinary [2] chondrites, and our recent modifications to reduce the desorption laser spot size from 1 mm to 40 \(\mu\)m has allowed us to expand this technique to extra-terrestrial bodies of limited size and fine spatial heterogeneity. We have recently used L\textsuperscript{2}MS to analyze the acid (HF-HCl) insoluble residues of the interiors of the carbonaceous chondrites Murray and Murchison, the unequilibrated ordinary chondrites Bishunpur and Semarkona, and the ordinary chondrites Saratov and Barwell. These residues, consisting of particles no larger than 200 \(\mu\)m in diameter, were individually mounted onto a sample platter for analysis.

In the L\textsuperscript{2}MS technique, the output of a pulsed CO\textsubscript{2} laser (10.6 \(\mu\)m) is focussed through a microscope objective onto the sample producing the rapid desorption of neutral molecules; the effective desorption region is a spot 40 \(\mu\)m in diameter. After a 19\(\mu\)s time delay, the frequency-quadrupled output of a pulsed Nd:YAG laser (266 nm) selectively ionizes the PAHs in the mixture of desorbing species by 1+1 REMPI. The ions are mass analyzed in a reflectron time-of-flight (TOF) mass spectrometer and detected by a microchannel plate.

Consistent with previous bulk chemical analyses, large PAH concentrations are found in the unequilibrated chondrites and undetectable amounts are found in the more equilibrated, thermally processed chondrites [3]. The acid residue of Bishunpur has the strongest signal (figure 1), followed by Semarkona, Murchison, Murray, and Saratov. The incremental spacings of 14 amu indicate the successive alkylated PAH skeletons. No detectable signal is observed from Harwell, which is consistent with its type-6 petrographic classification. This sample also serves as a reference for contamination introduced during sample preparation. Although great care was taken to ensure that we did not introduce contamination in our lab, the indigeneity of the organic material remains speculative, as the histories of the meteorites on Earth are not well documented.

In an effort to further classify the organic material, we have noted trends in the ratios of a set of PAHs in the meteorites. The ratios compare nicely between the Murchison and Murray samples and between the Bishunpur and Semarkona samples, while showing distinct differences between the carbonaceous and unequilibrated chondrites samples; reassuringly, these ratios differ from the other meteorite residues.

We also present an investigation of the laser induced desorption process. To better understand this process we are conducting experiments to quantitate the flow of energy from the laser heated substrate into the adsorbate molecules. Various coverages of naphthalene on a quartz substrate have been studied in the collision-free environment of an ultrahigh vacuum (UHV) chamber. Again, desorption is induced with a CO\textsubscript{2} laser.
(10.6 μm) and the molecules are ionized with the Nd:YAG laser (266 nm); however, the mass analyzer on the UHV apparatus is a linear TOF system. The energy transfer is described by the correlation of three experiments: 1) the time-dependence of the surface temperature, 2) the kinetic energy, and 3) the internal energy distributions of the desorbed molecules. We have successfully modelled the temperature evolution of the quartz surface using a numerical simulation program whose results agree to within 10% of experimental measurements [4]. We have also measured velocity distributions of submonolayer coverages of naphthalene; a Maxwell-Boltzmann fit to these distributions indicates a translational temperature of 224 K [5]. The last of these measurements is currently in progress.


**Figure 1:** Mass spectrum of the Bishunpur acid-insoluble residue. The labelled masses match those of bare PAH skeletons, except the peak at mass 152 which has been verified as a fragment of mass 178. The spectrum is a 15-shot average.
THE DESIGN AND PERFORMANCE OF A LASER DESORPTION -
LASER IONIZATION - TIME-OF-FLIGHT SPECTROMETER:
HOW TO DO IT YOURSELF.

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Facility for Advanced Instrumentation
University of California, Davis, CA 95616

Laser Desorption - Laser Photoionization - Time-of-Flight Mass Spectrometry (LD-LP-TOFMS) is aptly suited to environmental analyses due to the characteristics which have given it wide application from the study of small molecules to the characterization of biopolymers. The laser desorption step due to its insensitivity to salts and essentially instantaneous volatilization of the sample is an effective means to directly analyze soot or fly ash samples for polycyclic aromatic hydrocarbons (PAHs) and their chlorinated analogs eliminating laborious and costly extraction methods. Photoionization allows one by careful choice of wavelength to selectively ionize the PAHs from the complex matrix of the soot particles. The time-of-flight spectrometer with its yet undetermined upper molecular weight limit and superior resolving capability when equipped with a nozzle expansion and reflectron is the mass analyzer of choice for this pulsed source experiment. Since commercial instruments of this type were not readily available and funds were limited at project inception, we chose to design and build the instrument ourselves.

We designed and built a LD-LP-TOFMS outfitted to operate in linear or reflectron mode with the option of sample introduction / seeding via supersonic expansion. The SIMION software package was used to model the electrostatic field and trajectories of ions in the source and flight tube, both to sort out working designs for the construction phase and source parameters for focusing the ion beam while bringing the instrument on line. To date we have used the instrument in linear mode only with single step UV desorption / ionization and applied it to the characterization of our PAH standards. Several spots obtained from incineration experiments were analyzed to demonstrate the potential to obtain selective ionization of components by varying the ionizing wavelength. The 266 nm (frequency - quadrupled output of the Nd:YAG ) light was passed trough a Raman shifter which generated Stokes (299 nm) and anti-Stokes (239 nm) shifted UV wavelengths for Photoionization . Figures 1 and 2 show spectra obtained from a trichloroethylene (TCE) flame soot using 266 nm and 299 nm. We obtained our first spectrum combining CO2 laser desorption with 266 nm ionization from a soot produced in a benzene/methane flame.

In the course of our experiments we learned a great deal about the critical effects of probe design on the signal observed. Probe orientation as well as the nature of the sample / probe interface were explored. Other investigators have also encountered probe effects and come up with some unique solutions i.e. a prism probe or a porous substrate on the probe surface to eliminate spatial effects on the initial ion packet distribution and shot to shot desorption yield variances. Figure 3 shows a photodesorption / ionization spectrum of pyrene resulting from penetration of the evanescent wave beyond the prism boundary.

This work has been supported by NIEHS Superfund Basic Research Grant ES-04699 and a contract from the California Air Resources Board.
Evaluation of Factors Contributing to the Accuracy and Reliability of the Mass Assignments In Matrix Assisted Laser Desorption Mass Spectrometry

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EXPERIMENTAL

Matrix assisted LASER desorption experiments were run on a time-of-flight mass spectrometer model VT2000 manufactured by VESTEC Corp. The more important operational parameters were:
- Wavelength: 355 nm
- Accelerating voltage: in most cases 30 kV
- Matrix: sinapinic acid
- Amount of analyte: ca. 1 pmol

Method of sample preparation is described in Ref. 1. Data are processed on a MicroVAX 3600 computer. Data processing software for the VAX station which makes use of the large storage space and high CPU speed has been developed in our laboratory. Generally, 5-20 individual spectra (called runs) were taken from a single sample preparation and stored in a single data file. Runs contained averages from a number of laser shots (typically 50). Each run could be smoothed, calibrated, and displayed on the screen or plotted on a graphics printer.

COMPARISON OF INTERNAL AND EXTERNAL CALIBRATION

Whenever internal calibration is possible it is superior to external calibration. However, internal calibration is not always possible, especially if the molecular weight of the calibration compound is far from that of the unknown compound. This often the case with samples in the 100 kDa range where no calibration compound of known mass is available presently. External calibration is a good alternative in these cases.

Table 1. compares external and internal calibrations at different accelerating voltages. The mass of cytochrome C was determined calibrated to porcine insulin externally and as well as internally, too. The resolution is also included in the table measured at the (M+ H)⁺ peak of insulin, as an indicator of the quality of the spectra. It is remarkable that the accuracy is one order of magnitude better with internal calibration whereas improvement in reproducibility is less significant (that is, the decrease of σ). Accuracy and reproducibility of the mass assignment seems to be independent of the accelerating voltage in the range of 20-30 kV.

EFFECT OF AVERAGING

Originally, the aim of averaging a number of LD mass spectra was to reduce signal-to-noise ratio for the high mass molecular ions. However, a systematic study reveals that not only the S/N ratio but also the accuracy and reproducibility of the mass measurements improves impressively with averaging the individual spectra as shown in Table 2. Note that both proteins can be ionized very easily and give a strong signal even for single laser shots. It is somewhat unexpected that averaging single shot spectra also increases the resolution. Taking 20-50 shot averages seems optimal from the point of view of
accuracy, reliability, and sample consumption as well.

<table>
<thead>
<tr>
<th>Accel. voltage (kV)</th>
<th>External</th>
<th>Internal</th>
<th>Resolution</th>
<th>σᵣ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Error in mass (%)</td>
<td>σ</td>
<td>Error in mass (%)</td>
<td>σ</td>
</tr>
<tr>
<td>20</td>
<td>0.25</td>
<td>3.16</td>
<td>0.015</td>
<td>0.66</td>
</tr>
<tr>
<td>25</td>
<td>0.19</td>
<td>1.66</td>
<td>0.036</td>
<td>1.13</td>
</tr>
<tr>
<td>30</td>
<td>0.19</td>
<td>2.50</td>
<td>0.017</td>
<td>1.32</td>
</tr>
</tbody>
</table>

**TABLE 1** Measurement of Cytochrome C (MW 12,359.1); calibration to porcine insulin. "Determined at the (M+ H)⁺ peak of insulin.

<table>
<thead>
<tr>
<th>Shots averaged</th>
<th>Error in mass (%)</th>
<th>σ</th>
<th>Resolution</th>
<th>σᵣ</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>0.28</td>
<td>9.29</td>
<td>198</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>0.12</td>
<td>6.13</td>
<td>230</td>
<td>24</td>
</tr>
<tr>
<td>20</td>
<td>0.033</td>
<td>1.18</td>
<td>250</td>
<td>11</td>
</tr>
<tr>
<td>50</td>
<td>0.017</td>
<td>1.32</td>
<td>225</td>
<td>11</td>
</tr>
<tr>
<td>100</td>
<td>0.023</td>
<td>1.65</td>
<td>224</td>
<td>15</td>
</tr>
</tbody>
</table>

**TABLE 2** Measurement of Cytochrome C (MW 12,359.1); calibration to porcine insulin. "Determined at the (M+ H)⁺ peak of insulin.

**COMPARISON OF THE EXPERIMENTAL AND THEORETICAL PEAK SHAPE**

Comparison of the theoretical isotope distribution with the experimental peak shape can partly explain the uncertainties in mass accuracy. We compared the isotope distribution and the measured peak shape of the molecular ion of porcine insulin. The resolution was high enough to see low mass adducts or loss of small fragments. It turned out that in this case peak broadening in the mass spectrometer is due rather to energy spread of the ions than to adduct formation or fragmentation. This finding, however, may not hold for larger molecular ions.

**REFERENCES**

While matrix assisted laser desorption mass spectrometry is capable of ionizing biomolecules in excess of 200,000 daltons, the low mass resolution of the time-of-flight mass analyzers employed limits the obtainable mass measurement accuracy. This is compounded by the preponderance of adduct species at higher mass which further degrade the apparent mass spectral resolution and cause a significant uncertainty in determining ion peak centroids. While mass resolutions of 300-500 (fwhm) can be estimated for low mass proteins such as bovine insulin (mw=5733.5), it becomes almost impossible to accurately determine instrumental resolutions at higher mass. This is due to the natural broadening of the isotopic peak distribution at higher mass and the increase in the various ion species surrounding the molecular ion peak ([M+H]+H)\(^+\) at higher mass. Despite the problem with estimating mass resolution at \(m/z\) > 10,000, some degradation in experimental mass resolution seemed to be occurring at higher masses. This could be due to the presence of additional unresolved ion species not observed at lower mass which can cause an apparent loss of mass resolution or it could be the result of a physical effect which actually degrades instrumental mass resolution. The latter may be associated with the mechanism of very large ion detection as discussed by Spengler et. al.\(^1\). In order to determine the actual experimental mass resolution at higher masses, a computer program was developed to calculate the expected theoretical isotopic mass distributions for a given empirical formula and output a simulated peak profile for any given mass resolution. The program was written in FORTRAN and utilizes a variation on the algorithm described by Yergey\(^2\), optimized to run on an AT-clone personal computer. The output can be directly compared to experimental data by importing the output into our mass spectrometer’s data analysis program (Spectra Calc, Galactic Industries). Output for various ion structures can also be summed to produce a theoretical mass spectrum at a given mass resolution which can then be compared to experimental data.

Low mass peptides and proteins (<6,000 daltons) were run experimentally by matrix assisted laser desorption (sinapinic acid matrix; 337 nm laser wavelength) in order to identify the ion species produced. Most of the major ion species are easily mass resolved in this molecular weight range. In addition, two data processing techniques were employed to improve the apparent mass resolution of the data. Initially, spectral deconvolution of the spectra was employed but resulted in only minor improvement for lower mass species. Spectral deconvolution is a widely used technique, particularly in optical spectrometry, which can remove various line-broadening components of spectra. At higher masses, significant resolution improvement can be seen. Indeed, preliminary results suggest that this type of data processing can also improve mass accuracy determinations. The mass accuracy for horse heart cytochrome C (mw=12360.1) calibrated with myoglobin (mw=16951.5) improved from 0.01% to 0.005% when deconvolution was applied prior to time-to-mass conversion. For lower mass ions, maximum likelihood calculations provided significantly improved spectral resolution. The maximum likelihood method (Spectrum Square Associates) is a statistical process which finds the most likely spectral representation given a peak shape (gaussian) and estimated peak-width (fwhm). The results of this approach for the B chain of bovine insulin (mw=5349.95) are shown in Figure 1. It can be seen that the matrix addition peak actually corresponds to more than one ion species produced. These results are clearly observed, even in the raw experimental data (Figure 2) and are not an artifact of any of the spectral data processing techniques. The identified ion species observed for low mass proteins and peptides are summarized in Table I.

Using this information and the observed tendency of larger proteins to add multiple matrix species, theoretical peak profiles were constructed at various mass resolutions and fitted to the experimental data in an iterative approach. Using the peaks and relative intensities shown in Table 2, a best-fit theoretical mass spectrum for myoglobin was constructed based upon a resolution of 225 (fwhm) and is shown in Figure 3. The experimental and difference spectra are also shown. This compares to an experimentally measured resolution of 300 (fwhm) for bovine insulin. Based upon these results it appears that some of the resolution loss at higher mass is due to something other than additional ion species. The postulated acid (acetic and sulfurlc) adduct ions are consistent with species observed for lower mass proteins although they are not always observed for lower mass proteins and are never observed as multiple adds at low mass. Still, there existence is possible, given the known tendency for multiple matrix adds to...
(with significantly higher relative intensities than observed for lower mass proteins) in higher molecular weight proteins. Some ion species in addition to the known matrix adducts must be present in order to account for the observed experimental ion distributions. Future experiments include substituting trichloroacetic acid in the sample preparation for acetic acid to look for a mass shift in the adducts formed and chemical means to precipitate sulfate will be tried in an attempt to verify the sulfuric acid adduct.

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Table 1. Observed Low Mass Protein Ion Species Intensities As % Of [M+H]⁺

<table>
<thead>
<tr>
<th>Peak</th>
<th>Bovine Chain A</th>
<th>Bovine Chain B</th>
<th>Bovine Insulin</th>
<th>Porcine Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>[M+H⁺]⁺</td>
<td>5.74</td>
<td>4.16</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>[M+Na⁺]</td>
<td></td>
<td>0.117</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[M+H⁺+44]⁺</td>
<td>14.4</td>
<td>2.05</td>
<td>4.08</td>
<td>3.20</td>
</tr>
<tr>
<td>[M+H⁺+25]⁺</td>
<td>16.7</td>
<td>10.2</td>
<td>17.1</td>
<td>11.2</td>
</tr>
<tr>
<td>[M+H⁺+41]⁺</td>
<td>4.92</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>[M+H⁺+68]⁺</td>
<td>*</td>
<td>8.57</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>[M+H⁺+180]⁺</td>
<td>8.11</td>
<td>6.97</td>
<td>14.3</td>
<td>13.6</td>
</tr>
<tr>
<td>[M+H⁺+206]⁺</td>
<td>3.80</td>
<td>3.28</td>
<td>11.4</td>
<td>10.8</td>
</tr>
</tbody>
</table>

Note: [M+H⁺+209]⁺ is an unresolved composite of peaks [M+H⁺+180]⁺; [M+H⁺+206]⁺; [M+H⁺+224]⁺ with relative ratios determined from bovine insulin.

References:
Electron and Ion Conversion From Large Molecular Ions Incident on Various Surfaces

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Introduction: Detection of small ions in time-of-flight (TOF) mass spectrometry normally requires the ejection of electrons from a surface, followed by electron multiplication, then current measurement or pulse counting. Electron conversion is a convenient mechanism for ion detection in TOF measurements because the electrons have a high velocity compared to the ions, so they produce little time dispersion. However, the efficiency of electron production depends on the velocity of the incident ion; it drops to a very low value below a “threshold” velocity $\approx 10^6$ cm/s [1]. Thus, detection of large ions by this process is difficult without application of very large accelerating voltages to the incident ions.

Evidence has been accumulating that detection of large molecules is accomplished mainly by secondary ion emission, rather than electron emission [2,3]. In order to investigate this question and to determine the properties of various surfaces as secondary electron or ion emitters, we have constructed a tandem TOF system where large molecular ions produced by matrix-assisted laser desorption are used as primary ions.

Experimental: A schematic diagram of the apparatus is shown in Figure 1. A wide range of molecular and cluster ions were produced by matrix-assisted laser desorption using sinapinic acid as a matrix. After mass selection in one TOF tube, they were incident on various surfaces. The ions produced at these surfaces were accelerated and mass analyzed in a second TOF tube. Positive laser desorbed projectiles of 13 keV or 15 keV were used. Normally, the potential of the investigated surface was $V_g = -5$ kV or $-8$ kV, (i.e. negative ion spectra were observed), but some spectra of positive ions were also taken using $V_g = +5$ kV. The resolution in the secondary spectra is limited by the time spread of the primary ions, which becomes larger for higher molecular weight projectiles.

Electron Conversion and Large Molecules: Our results confirm earlier findings that for large molecules incident on surfaces, ion conversion dominates electron conversion. For 23 keV insulin molecular ions incident on a CsI surface, only a small shoulder due to electrons is observed on the H$^-$ peak. This shoulder disappears when a magnetic field is applied. For molecular ions of trypsin, the magnetic field has no discernible effect on the secondary spectrum. In these measurements, a two grid system was used to prevent ions that originate at the grid from striking the target.

Secondary Ion Spectra: Detailed analysis is still in progress but the following observations can be made:

a) For incident projectiles of molecular weight greater than $\approx 5000$ u, the secondary spectra between m/z 10 and 80 are remarkably similar, independent of surface (see Fig. 2). The dominant ion in this range is at m/z 25, presumably CpH$^-$.

b) The H$^-$ ion falls off in intensity with respect to the larger ions as the projectile mass is increased. However, a signal from H$^-$ is observable for projectiles at least up to the monomer of albumin, and possibly for its dimer at mass 132 000 u.

c) Although an 18 keV insulin ion moves relatively slowly (about the speed of a 3 eV proton), it can cause the ejection of small intact molecular ions (Tyr-Gly) from surfaces.

The Mechanism of Ion Conversion: The similarity of the secondary ion spectra from insulin bombardment of different surfaces (above) suggests that the ions are produced by surface-induced dissociation. This interpretation is supported by the difference from the spectra obtained with a light projectile like benzopyrene (276 u), where desorption is the main process (Fig. 3).

Further evidence for surface-induced dissociation was obtained for positive secondary ion spectra from a CsI coated surface. As the mass of the projectile increases, the abundance of the Cs$^+$ ion desorbed from the surface decreases relative to the ions below m/z 100 (Fig. 3). For projectiles above mass $\approx 10 000$ u, no Cs$^+$ signal was observed. Since the relative H$^+$ yields also drops with increasing projectile mass, it may be partly accounted for by desorption from the surface.

Conclusion: For molecular ions above 10 000 u incident on a surface at $\approx 20$ keV, electron conversion is insignificant compared to ion conversion. The secondary H$^+$ yield also decreases with projectile mass, but H$^-$ ions are still observed for projectiles up to at least 66 000 u.

The distribution of secondary ions is sensitive to the nature of the surface for primary ions larger than insulin, suggesting that surface-induced dissociation is the main process of ion conversion.

The dominant negative secondary ion is at m/z 25 while the positive distribution peaks at about m/z 60.

Excimer Laser beam (308 nm)

Fig. 1 Schematic diagram of the apparatus.

Flight Time (ns)

Fig. 2 Negative spectra from CsI for various projectiles

Flight Time (ns)

Fig. 3 Positive spectra from CsI for various projectiles

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This report discusses research directed towards optimizing and understanding laser desorption (LD) of neutral molecules. A variety of compounds were desorbed at the four harmonic wavelengths of a Nd:Yag laser (1064, 532, 355, and 266 nm). The jet entrained neutrals were post-ionized with 255 nm Multiphoton Ionization (MUPI) and mass analyzed with a RETOF MS. In principle, the advantage of separating the LD step from the ionization step is that each step can be independently optimized to yield improvements in system performance while allowing greater flexibility in both ionization and desorption conditions. In addition MUPI mass spectra are relatively easy to interpret since post-ionization of desorbed neutrals leads to the formation of pure molecular ions without adduct ion formation [1]. Finally separation of desorption and ionization allows direct probing of the significance of resonant sample absorption for efficient LD by investigating the neutral sample desorption efficiency as a function of desorbing wavelength and sample absorption.

In the first study the effect of sample absorption at the desorbing wavelength was investigated. Lower LD threshold energies were observed under resonant LD conditions, as shown in Table 1. The results also showed that resonant LD efficiently produced abundant neutral molecular species from all compounds studied [2]. Under non-resonant LD conditions no intact neutral molecules could be detected for the thermally labile compounds and these results suggested that substrate heating does not lead to desorption of intact molecules from thermally labile compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wavelength of Desorbing Laser</th>
<th>Irradiance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>266 nm</td>
<td>355 nm</td>
</tr>
<tr>
<td>Pentacene</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>(21000)*</td>
<td>(1000)</td>
<td>(5000)</td>
</tr>
<tr>
<td>Coumarine</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>(5000)</td>
<td>(9000)</td>
<td>(0)</td>
</tr>
<tr>
<td>Ala-Trp</td>
<td>7</td>
<td>61 (Fe')</td>
</tr>
<tr>
<td>(4400)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>Leu-Phe</td>
<td>14</td>
<td>220 (Fe')</td>
</tr>
<tr>
<td>(150)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
</tbody>
</table>

* All values given in this table are observation thresholds.
* Numbers in parentheses are methanolic solution absorptivities in L/mol·cm.
* Pentacene values measured in benzene.
* Underlined thresholds were measured using focused LD conditions.
° (Fe') indicates that appearance of the molecular ion signal was observed to coincide with the observation of post-ionized substrate specific Fe neutrals.
* N.S. indicates no signal from post-ionized neutrals was observed.
In the second study 266 nm resonant LD of a number of peptides up to a mass of 2000 da was performed. Variations in the ionizing laser power and wavelength strongly indicated that formation of neutral sequence specific fragments occurs during the LD process. Both N-terminal fragments ($A'$, $C'$) and C-terminal fragments ($X_n$, $Y_n$, $Z_n$) were observed. As expected for neutral fragments formed during LD, only MUPI ionizable chromophore containing fragments were observed. These fragments can be useful in determining the amino acid sequence of peptides, as shown for Substance P in Figure 1.

![Fig. 1: 266 nm Laser Desorption of Substance P. The desorbed neutrals were ionized at a wavelength of 255 nm.](image)

In the third study absorbing matrices were added to assist the desorption of thermally labile compounds at non-resonant wavelengths. Beside the metal matrices Pt, Au and Ag, other absorbing matrices like Pentacene and Graphite could be used to enhance desorption of neutral molecules from non-absorbing compounds. Efficient laser desorption of thermally labile intact neutral molecules is only achieved by direct coupling of the desorbing laser energy into the sample bulk via absorption by the sample itself or by a dispersed absorbing matrix.

**Literature:**


**Acknowledgement:**

This work was supported by grants from the Bundesministerium für Forschung und Technologie (13N5307) and the Deutsche Forschungsgemeinschaft (Gr 917/1-2). Dr. G.R. Kinsel wishes to gratefully acknowledge the fellowship support of the Alexander von Humboldt Stiftung-Bonn, Germany.
Recombinant human macrophage colony stimulating factor (rhM-CSF) is a hematopoietic growth factor that induces proliferation of cells of the monocyte/macrophage lineage. The CHO-expressed protein is a homodimer consisting of two 223 amino acid disulfide linked subunits which result from processing of a 553 amino acid precursor. In addition to extensive N- and C-terminal processing, each monomer is modified via the addition of two sites of N-linked glycosylation and several sites of O-linked glycosylation. Based on extensive mass spectrometric characterization of the monomeric subunits, the major species of native rhM-CSF have chemical molecular masses of 64-66 kD. Nonreducing SDS-PAGE yields an apparent molecular mass of 90 kD. This large discrepancy is due to the fact that migration in the SDS-PAGE system is based upon the physical properties of the protein. In addition, SDS-PAGE may be insensitive to small changes in mass due to the action of proteolytic or glycolytic enzymes.

The recent commercialization of LD-TOF offers an alternative to SDS-PAGE for the characterization of molecules produced by recombinant technologies. LD-TOF measures the chemical mass of the molecule and thus is unaffected by the physical/hydrodynamic properties of the protein. In addition, with reported mass accuracy of 0.1%, shifts in molecular mass may be assessed and assigned to specific modifications. Based on these features we have developed a strategy (Scheme I) which can provide structural information during the early stages of cell culture or purification. This method involves chemical or enzymatic manipulation of the molecule interspersed with molecular mass analysis. As an example, rhM-CSF was analyzed in its native state and after various manipulations. Analysis of native rhM-CSF by LD-TOF results in a broad peaks in the 64-66kD range due to the various sites of glycosylation within the dimer. Reduction and alkylation of rhM-CSF to produce monomeric rhM-CSF also yields a broad peak. This peak broadening may be a result of the contribution of the number of different carbohydrate forms of rhM-CSF. Removal of the C-terminus of dimeric rhM-CSF reduces the mass, however, the peak is still broad due in part to the remaining N-linked carbohydrate in the N-domain. Reduction and alkylation of this species yields monomeric N-domain and results in a slightly sharper peak in which at least two species are partially resolved. Finally, removal of the N-linked carbohydrate from this sample results in one very sharp peak and another partially resolved peak shifted up in molecular mass. The shift in mass may correspond to the presence of a single site of O-linked glycosylation. A comparison of these LD-TOF spectra with the same species run in nonreducing and reducing SDS-PAGE gels illustrates some of the advantages of LD-TOF MS. Based on these results as well as the increasing number of literature references, it is apparent that LD-TOF is a powerful addition to the analytical tools employed for the characterization of recombinant proteins (Scheme II). A few of the potential applications of LD-TOF mass spectrometry in the field of biotechnology include; accurate molecular mass determination of intact proteins, evaluation of posttranslational processing events or posttranslational modifications, assessment of protein heterogeneity due to proteolysis or incomplete processing, disulfide bond analysis, assessment and monitoring of protein integrity following certain process or purification steps, rapid characterization of a protein isolated from cell culture media without the need for extensive purification and the study of receptor/ligand interactions or receptor/ligand complexes from chemical cross-linking experiments.

In order to process LD-TOF data obtained in different laboratories a general PC based peak detection and processing software package has been developed. The program incorporates a set of flexible commands, employs mouse operation and can process laser desorption and electrospray ionization mass spectra. It contains a series of algorithms which effectively address several characteristic features of LD-TOF spectra including; broad, unresolved peaks, poor signal-to-noise and peak shapes and irregular baselines.

Acknowledgements: We gratefully acknowledge the time and effort of Dr. Marvin Vestal and Dr. Randall Nelson of Vestec Corporation for providing instrument time and expertise in acquiring the spectra described above.
Scheme I
Strategy for the Structural Characterization of Recombinant Glycoproteins

- Recombinant Protein
  - Specific Degradation
  - Complex Peptide Mixture
    - RPLC
    - LD-TOF

- Structural Characterization of Peptides
  - Mass Spectrometry
  - MS-Toasted Sequence Analysis
    - Amino Acid Composition

Primary Structure of Glycoprotein

Scheme II
Analytical Tools for the Characterization of Recombinant Proteins

- Characterization Tools
  - Bacteriophage Mammalian Expression of the Protein
    - SDS-Page
    - LD-TOF (molecular weight)

- Purification of the Protein
  - SDS-Page
  - N-Terminal Analysis
  - Amino Acid Composition
    - LD-TOF (molecular weight)

- Structural Characterization of the Protein
  - Two-Sector MS (molecular weight)
  - Four-Sector MS (sequence)

Level of Structural Detail
- Low
- High
Mass Measurement Accuracy in
Matrix Assisted Laser Desorption Mass Spectrometry

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Matrix Assisted Laser Desorption Mass Spectrometry provides a rapid and sensitive means of obtaining molecular weight information from biologically important molecules such as proteins and carbohydrates. In most cases, mass analysis has been by Time-of-Flight (TOF) which provides excellent sensitivity and mass range but only limited mass resolution.

Factors which cause loss of resolution by peak broadening are less of a concern than those which cause a mass measurement error by shifting the peak centroid. Resolution is useful in mixture analysis, but this requirement can often be circumvented by prior use of chromatography. A working resolution of 500 to 1000 would probably suffice in most cases.

The greatest source of peak broadening and mass measurement error in Matrix Assisted LD is undoubtedly adduct formation [1].

The most prominent adduct observed in protein spectra is between the sample and the preferred matrix degradation product. In the case of sinapinic acid, this adduct gives rise to a satellite peak at (M+206). If this main adduct peak cannot be resolved from the sample peak then a substantial mass measurement error is introduced. The relative intensity of the adduct peaks tend to increase with mass so that, even if the main adduct peak cannot be resolved, the use of an internal calibrant which is sufficiently similar to the unknown as to exhibit a similar level of adduct formation will provide a partial correction.

It has been widely reported that increasing the laser power much over the threshold for ion production causes a dramatic broadening of the peakshape. This broadening is asymmetric, being mainly on the high mass side, so that the result is a mass deviation in addition to a reduction in resolution. It is likely that the several factors are responsible for the observed phenomena. We suspect that the main contribution comes from an increased degree of adduct formation, particularly the addition of one or more alkali metal atoms.

Other contributions to peak broadening and shifting are much less important than adduct formation. Ion - neutral collisions close to the sample surface are a possible cause of tailing on the high mass side of a peak. Significant levels of metastables have been observed using a reflectron mass analyser [2]. On linear instruments, the effect of metastable decay is limited to peak broadening caused by the release of kinetic energy during fragmentation.

Published data on measured energy spreads indicate that initial kinetic energies are low, in the region of 2 eV [3]. Even allowing for this to rise with increasing irradiance, it seems unlikely to be a significant contributor to the observed peak width.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

The mass measurement accuracy which can be achieved without an internal standard is especially important. Many additional sources of error influence external calibration. Sample to sample variations in the length of the flight path, the energy of the ions, and the inherent precision of the timing electronics will appear as deviations in measured mass.

By careful attention to instrument design, it is realistic to expect a mass measurement accuracy of 0.1% without the use of an internal standard. Under favourable conditions, internal calibration can give an accuracy approaching 0.01%.

Examples for recent progress in matrix-assisted laser desorption/ionization (LDI) will be described in the following, for the ultraviolet (UV) range (A) and the infrared (IR) range (B):

(A) A series of experiments has revealed that 2,5-Dihydroxybenzoic acid (DHB) represents a matrix compound with excellent performance at wavelengths of 337 nm (nitrogen laser) and 355 nm (tripled Nd-YAG wavelength). Part of the advantages observed for DHB is based on a pronounced crystallization, if small volumes (0.1-1 μl) of solutions containing matrix and analyte are dried slowly. LDI from large crystal needles protruding towards the insight of the preparation shows high shot-to-shot reproducibility and allows for at least several hundreds of successive shots on the same spot at threshold irradiance without deterioration of the macromolecule ion signal. This indicates a very low (some tens of nm) ablation depth and an extremely low (in the attomol range) amount of analyte consumed for a mass spectrum. Further advantages of a DHB-matrix are lack of/low intensity adduct ion formation and a relatively clean and non-intense matrix background (below 300 Dalton). DHB can be used both for proteins, native and derivatized oligosaccharides, as well as for more complex glycoconjugates as glycosphingolipids; it can be applied over a wide range of protein concentrations (10⁻⁵ to 10⁻³ M), thus a sensitivity in the low fmol range (total sample amount loaded) is easily attainable. Furthermore DHB also exhibits a high tolerance for even high concentration impurities and biochemical reagents, such as inorganic salts, buffers and detergents, including SDS (sodium dodecysulfate). Figure 1 shows a matrix UVLDI mass spectrum of a hemoglobin sample containing 10% SDS, taken at 337 nm and accumulated from 20 single laser shots.

For a systematic investigation large (mm-size) crystals have been grown from saturated DHB solutions containing low concentration of proteins. Protein molecules are homogeneously distributed within the matrix host crystals as can be controlled by LDI from both crystal surface and its inside, the crystals even show the diffraction pattern of an undisturbed DHB single crystal in preliminary X-ray crystallography experiments. This intimate mixture of matrix and analyte molecules (but not of low molecular weight impurities) is therefore regarded to be the reason for the superior performance of these crystals, but also of that of crystal needles in regular preparations in LDI.

Until now matrix-LDI mass spectra have generally shown low mass resolution. Best results (mass res. of ≤ 500 FWHM) have surprisingly been obtained with linear TOF instruments using high initial ion acceleration. In the meantime it has been shown that peak broadening due to adduct ion formation, time spreads induced by secondary ions instead of electrons in the detection process and possibly metastable fragmentation of macromolecular ions may explain the observed large peak widths. Improved reflector-type TOF mass spectrometers have recently been installed proving that mass resolutions of more than 2000 are accessible also for matrix-LDI conditions of peptides and small proteins. Registered peak widths of less than
7 ns close to the pulse width of the laser indicate that there are no principal limits for the attainable mass resolution as supposed by delayed ion formation in the gas phase. Figure 2 shows a mass spectrum of porcine cytochrome C, measured with a reflectron TOF of 1.7 m equivalent drift path. The observed peak width of 10 Dalton (see extension) is close to the envelope of the isotopic distribution. A lower intensity peak due to the loss of water is also observed. This high mass resolution capability may become important for an improvement in mass determination accuracy, because peak components formed by cationized analyte molecules and adduct ion species can be sorted out; it will also increase the ability to determine chemical heterogeneity of compounds.

(B) The current experience for matrix-assisted Infrared LDI indicates that most of the features are comparable to those observed in the UV range. Experiments were performed with pulsed Er-YAG (2.94 μm), Er-YSGG (2.79 μm) and with a CO₂ laser (10.6 μm). Preparation requirements, accessible mass range and tolerance of even high concentration impurities are similar, a pronounced influence of the laser wavelength/matrix absorption properties is also observed. Differences appear in a smaller shot-to-shot reproducibility (except for liquid matrices) and a higher amount of ablated material. Furthermore, a tendency for the formation of more highly charged molecular ions becomes apparent, strongly pronounced for some matrix compounds. Advantages are a considerably larger number of usable matrices covering very different classes of organic compounds, as well as the availability of several liquid matrices (glycerol, triethanolamine and lactic acid). Most promising are initial results proving that Tris-buffer and especially water (by freezing down aqueous protein solution) are working as a matrix in IR-LDI. Figure 3 and 4 show spectra of cytochrome C and lysozyme taken from a dried protein solution in Tris-buffer and a small volume of a frozen aqueous protein solution respectively.

Figure 2: Matrix UV-LDI mass spectrum of porcine cytochrome C; DHB-matrix, 355nm, accum. from 10 single laser shots

Figure 3. Matrix IR-LDI mass spectrum of porcine cytochrome C, matrix: Tris-buffer pH 7, 2.94 μm, accum. from 20 single laser shots

Figure 4. Matrix IR-LDI mass spectrum of lysozyme from chicken egg, matrix: ice (frozen aqu. solution), 10.6 μm, accum. from 15 single laser shots

Laser Desorption (LD) and Plasma Desorption (PD) Mass Spectrometry have been used as off-line detectors for various forms of chromatography and electrophoresis. We routinely characterize isolates from column, thin layer and high-performance liquid chromatography. We have also developed simple approaches for collecting fractions from both supercritical fluid chromatography and capillary zone electrophoresis. Finally, we have developed initial methods for recovering proteins from SDS-polyacrylamide gel electrophoresis.

A reverse-phase HPLC chromatogram of an Olestra sample, prepared by reacting sucrose with a mixture of C\(_2\), C\(_4\), C\(_6\) and C\(_8\) fatty acids, is given in Fig. 1 (top). A total of 2 mg of sample was injected and seven fractions were collected as indicated. Excellent-quality mass spectra were obtained on all of the fractions, including fractions 3, 5 and 7. The spectrum of fraction 7 is given in Fig. 1 (bottom). It was obtained by PDMS analysis of about 1 µg of total mass. Given that about 10 major components were detected, it is clear that acceptable quality PD mass spectra can be obtained on about 100 ng of non-polar, high mass molecules. These materials would be difficult to analyze with on-line methods such as flow-FAB and electrospray ionization.

The experimental arrangement that we are currently exploring for SFC/PDMS is given in Fig. 2 (top). Analytes are detected using a non-destructive in-line UV cell. After detection, analytes which are normally vented to atmosphere are simply intercepted with a PDMS sample foil. The fraction of the eluant that sticks to the surface is then available for MS analysis. SFC peak widths are typically broad enough that it is easy to collect fractions even when they elute adjacent to one another in the chromatogram. Fig. 2 (middle) shows a supercritical fluid chromatogram of a commercial nonionic surfactant mixture. Eight fractions were collected from a single injection of this mixture. The PD mass spectrum of a representative fraction, indicated with an arrow, is given in Fig. 2 (bottom). Excellent-quality spectra were obtained with only a few minutes of sample integration time. Off-line PDMS detection limits for nonionic surfactants are presently about 5 ng (injected onto the SFC). We are currently attempting to develop SFC collection methods that are compatible with the use of flame ionization detectors.

The approach used to collect fractions from CZE employs a porous glass joint near the cathodic end of the separation column (1,2). We have previously demonstrated sub-picomolar PDMS detection limits for small synthetic peptides collected from CZE(2). Initial results obtained using matrix-assisted laser desorption mass spectrometry are also very encouraging. The LD mass spectrum of 1.5 pmol of a bovine trypsinogen isolate (a total of 3 pmols of analyte was initially injected onto the column) is given in Fig. 3. Even larger proteins such as bovine serum albumin have been successfully collected and analyzed by LDMS.

We are currently using electroelution to facilitate the recovery of large proteins from SDS-PAGE. Protein recoveries, determined in separate quantitative HPLC experiments, are in the range of 30 – 40%. A LD mass spectrum of carboxymethylated lactalbumin, electroeluted from an SDS-polyacrylamide gel after electrophoresis, is given in Fig. 4. The signal-to-noise ratio is fairly weak, possibly because SDS quenches LDMS response(3). In the example shown, we simply used a hydrophilic membrane to retain the protein, while SDS and other low-molecular-weight impurities were washed through. Overall sensitivity will hopefully increase as improved methods for SDS removal are developed.

Fig. 1 Reverse-phase HPLC chromatogram of Olestra (top); PD mass spectrum of fraction 7 (bottom).

Fig. 2 Experimental arrangement for offline SFC/PDMS (top); chromatogram of a commercial nonionic mixture (middle); PD mass spectrum of the indicated component (bottom).

Fig. 3 LD mass spectrum of 1.5 pmols of bovine trypsinogen isolated from CZE.

Fig. 4 LD mass spectrum of carboxymethylated lactalbumin electroeluted from an SDS-polyacrylamide gel.
CO$_2$ laser desorption of organometallic complexes in the source cell of an FTMS-2000 dual cell FT-ICR (Extrel, Madison, WI) gives dramatically different product ions in the two cells of the instrument. In the source region we observe the parent ion and low energy fragments ions. In the analyzer region the bare metal ion is the base peak. (Figure 1) The intensity of the ions in the analyzer region are 10-50 times greater than the ion in the source region. The partitioning of the laser desorption products is not observed to depend on the trapping voltages or the voltage on the conductance limit. Even with 10 V on the conductance limit we still observe the atomic metal ion in the analyzer region. This phenomenon is observed for both neutral complexes such as Re$_4$H$_4$(CO)$_{12}$ and ionic complexes such as [Bu$_4$N]$^+$Pd[dmit]$^-$. The partitioning of the products does appear to depend on the laser focus and energy. When the beam focus was changed the intensity in the analyzer region was significantly reduced and in some cases the metal ion was not observed. The dependance of the partitioning of the product ions on laser focus and power is still under investigation.

Re$^*$ (IP = 7.76 eV) in the analyzer region reacts by charge transfer with NO (IP = 9.26 eV) added through a pulse valve. This reaction is not observed to go to completion but it indicates that at least some of the Re$^*$ in the analyzer region is in an excited state (Re$^*$a$^3$D = 9.47 eV). The reaction is slow with an apparent rate constant on the order of 2x 10$^{-11}$ molecules cm$^3$ s$^{-1}$. Re$^*$ reacts with aromatic molecules by dissociative addition. Benzene reacts with Re$^*$ by successive dehydrogenation steps;

$$\text{Re}^* + C_6H_6 = \text{ReC}_6H_4^+ + H_2$$
ReC₆H₄⁺ + C₆H₆ = Re(C₆H₄)₂⁺ + H₂
Re(C₆H₄)₂⁺ + C₆H₆ = Re(C₆H₄)₃⁺ + H₂

Also observed were Re(C₆H₄)(C₆H₆)⁺ and Re(C₆H₄)₂(C₆H₆)⁺ and at long reaction time these clusters became dominant. Re⁺ also reacts to dehydrogenate toluene.

This partitioning phenomenon is a way to easily produce atomic metal ions of reactive metals or radioactive metals such as ⁹⁹Tc for ion molecule reaction studies.
AN ELECTROSTATIC ENERGY ANALYZER TIME-OF-FLIGHT INSTRUMENT FOR FUNDAMENTAL STUDIES OF MATRIX-ASSISTED-UV-LASER DESORPTION IONIZATION

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The E-TOF/R-TOF instrument is shown schematically in Figure 1. The ions are extracted and focussed by a three element lens SIMS source. MS-I consists of a Kratos MS-9 electrostatic energy analyzer. MS-II consists of a multi-element reflectron with an angle of 0°(co-linear). Time-of-flight spectra can be obtained by using a time-to-digital converter (LeCroy model 4208 TDC), a 350 MHz digital oscilloscope (LeCroy model 9450), or a transient recorder (LeCroy model TR882D 200 MHz digitizer). All of the TOF data acquisition modules are interfaced to an IBM-AT(286) computer by using a National Instruments GPIB mainframe card. Event sequencing for the various experiments is controlled by a EG&G Princeton Applied Research digital delay generator model 9650.

The laser systems consist of 1) a Laser Science Inc. cartridge type N, laser (model VSG 337 at a 1-20 Hz pulse rate) with a pulse duration of 3 ns that is used for ionization, and 2) a Questek Series 2000 Excimer Laser (200 mJ(Ar/F₂, 193 nm) 1-80 Hz pulse rate) with a pulse duration of 8-17 ns that is used for photodissociation.

The reflectron TOF to be used for the E-TOF/R-TOF consists of a total flight path of 3.6 meters and the reflection angle is approximately zero degrees. The advantages of a colinear reflectron are high transmission and high mass resolution in comparison to that obtained with larger (5°-20°) reflecting angles. The colinear arrangement is also convenient for positioning detectors to detect both neutrals and ions. For example, neutrals forms by metastable ion dissociation reactions, collision-induced dissociation (CID), or photodissociation in the region between MS-I and MS-II are detected by a microchannel plate detector (detector #2) positioned behind the reflectron. Ions are detected by the microchannel plate detector (detector #1) positioned at the entrance to the reflectron TOF.

A particular advantage of the ion-neutral correlation experiment is the m/z resolution of MS-II is not determined by the temporal characteristics of the ionization process. For example, unimolecular dissociation, collision-induced dissociation, or photodissociation of m,* (mass-selected by MS-I) produces a fragment ion (m,*) and a neutral (m,0), and the velocity of m,0 and m,* is the same as that of m,*.

Neutral fragment will not experience any force due to the reflectron and will strike detector #2 and produce a signal. m,* will be decelerated upon entering the reflectron, eventually reaching zero velocity, at which point its direction is reversed. m,* is then re-accelerated back to its initial velocity. Because the initial velocities of the fragment ions is independent of the m/z value, the time that an ion spends in the reflectron depends upon the rate of deceleration and re-acceleration. The rate of deceleration/re-acceleration is proportional to the m/z of m,*.

The ion-neutral correlation experiment can be performed in two ways. The first is to use the neutral signal as the start for the time-of-flight measurement and measure the arrival time for the ion. The second method uses the ion as the start event for the time-of-flight experiment while delaying the signal from the neutral detector. The two methods should give identical spectra. A special requirement if this experiment is the need for a low beam current density in order to eliminate (or minimize) the number of false correlations.

The standard mode of operation for time-of-flight instruments is to form ions by using a pulsed ionization source, pulsed laser (for multiphoton ionization or desorption), and, assuming the ions are formed with a narrow distribution of kinetic energies, the mass resolution of the time-of-flight measurement is limited by the temporal characteristics of the ionization pulse. This is the reason that the ionization pulse durations of 1-3 nanoseconds are required for high mass resolution measurements. The use of short ionization pulses presents a major obstacle to performing laser ionization/laser photodissociation tandem mass spectrometry experiments. For example, the efficiency of single photon photodissociation is quite low ranging from a few per cent to 0.1-0.5% for large molecules. If we assume
a photodissociation efficiency of 0.1%, then $10^6$ primary ions yields 10 fragment ions. Because the number of reaction channels available to the activated ion is quite large (say 10-15 structurally significant fragment ions are typical for relatively small peptides), it may be necessary to accumulate data from $10^6$-10$^7$ events in order to collect a statistically significant data set. If the repetition rate of the laser is 10kHz, a data set of $10^6$ events can be obtained in 100 seconds. However, as the ion yield or photodissociation efficiency decreases, as is observed for increasing molecular weight, the number of events required to collect a statistically significant data set also increases.

An alternative approach to performing measurements with a pulsed ion beam is to form large numbers of ions per ionization pulse and collect the data by using a transient recorder. This is a common mode of operation for matrix-assisted laser desorption where the yield of ions per laser pulse is high, e.g., 10-100 ions are formed for each laser pulse. The limitation of this experiment is the high rate of sample consumption and the inherent loss in sensitivity over single ion counting experiments. The sample consumption rate does not effect acquisition of normal mass spectral data but this does pose a problem for signal-averaging. In addition, because the efficiency of the activation/dissociation process is low the transient recorder mode is less sensitive than TDC.

A complicating factor associated with laser desorption ionization, and especially matrix-assisted laser desorption, is the temporal evolution of ions following laser excitation. The laser pulse width is approximately 3 ns but the arrival-time distribution for the [M+H]$^+$ ions are bimodal. One portion of the arrival-time distribution extends to 50-70 us. This result complicates tandem mass spectrometry experiments with an instrument such as shown in Figure 1 in two ways: (i) the start signal for the time-of-flight experiment is not sufficiently defined to give acceptable mass resolution, and (ii) to achieve reasonable mass resolution it is necessary to pulse the ion beam entering MS-II such that a small slice of the arrival distribution is selected. Although such an experiment achieves the desired result, the sensitivity is reduced because a substantial portion of the ions produced are not analyzed.

Figure 1. Schematic diagram of the pulsed ion source/pulsed laser E-TOF/reflection TOF.
MEASUREMENT OF INITIAL TRANSLATIONAL ENERGIES OF PEPTIDE IONS IN LASER DESORPTION/IONIZATION MASS SPECTROMETRY

Y. Pan and R. J. Cotter, Middle Atlantic Mass Spectrometry Facility, Johns Hopkins School of Medicine, Baltimore MD 21205

Initial translational energy distributions of [Arg]-vasopressin and lysozyme molecular ions generated by matrix-assisted UV laser desorption/ionization were measured using a delayed ion extraction linear time-of-flight mass spectrometer. Energy distributions of the nicotinic acid matrix ions, with or without the presence of peptide, were also measured. These were compared with the energy distribution of gramicidin ions using IR laser desorption.

The principles of determining ion translational energy distribution using the delayed ion extraction TOF method have been derived in a previous study by Spengler and Cotter. A Q-switched frequency quadrupled Nd:YAG laser (Continuum YG660, Santa Clara, CA) was used for the UV laser experiments. A CO₂ pulsed laser (Tachisto 215A, Needham, MA) was used for the IR laser experiments. Ions were desorbed for a silver metal surface.

In a delayed ion extraction TOF experiment, ions formed during the short laser pulses (t=0) are extracted into the ion acceleration region at a later time (t=t₀). During the time t₀ ions drift in the field free region at their initial desorption velocities. The velocity components at direction normal to the desorption surface can be calculated as a function of t₀’s and the location of the probe. The ion velocities then can be directly inverted to the translational energies of the ions. The ion population at a particular delay time was measured from the ion signal intensity in the mass spectrum. The experimental method used here can be considered as a more direct way of measuring initial translational energies of ions. In contrast to ion retarding potential techniques or peakwidth analysis, the ion energies are determined independently of ion acceleration.

The initial translational energy distributions of both [Arg]-vasopressin (Figure 1) and lysozyme molecular ions (Figure 2) desorbing from the matrix mixture by UV laser irradiation markedly deviate from normal distribution. From the measurements shown in Figures 1 and 2, the molecular ions of vasopressin (m/z 1085) have typical translational energies of about 2 eV with 8 eV (FWHM) energy spread, the molecular ions of lysozyme (m/z 14300) have typical translational energies of 5-8 eV with 15 eV energy spread. The unsymmetrical ion populations at the high energy side is in contrast to the measured initial translational energy distribution of gramicidin molecular ions by IR laser irradiation as shown in Figure 3. The high energy ion population therefore may be attributed to the effect of the matrix ions and neutral molecules as a result of a collective desorption, of which the peptide ions may receive high translational energies by moving with small particles of high velocities. This become more evident as the speed distributions of both peptide and matrix ions were examined. Two laser irradiances were used for lysozyme energy measurements. No significant differences in measured translational energy distributions were observed as the laser irradiance increases from the threshold to a much higher irradiance. Increase in laser irradiance only increases the total ion yields (in this case it increases by 4 times) but does not change the translational energy distribution (Figure 2). This observation is in consistent with other studies.

The translational energy distributions of the nicotinic acid matrix ions are also worth noticing. (Figure 4). The energy distribution of nicotinic acid ions from the vasopressin/matrix mixture is basically the same as that of the pure matrix with a maximum ion population at about 1 eV. However the energy distribution of nicotinic acid ions from the lysozyme/matrix mixture shifts much to the lower energy side with a
maximum ion population at less than 0.2 eV. This decrease in translational energy of the matrix ions may be a result of collisions of the small matrix ions with large peptide ions as they move as packets.

Figure 1. [Arg] vasopressin/nicotinic acid mixture by UV/LDI. 1 ul 10^-6 M peptide aqueous solution and 1 ul matrix solution were applied to the silver probe and air-dried. (Sample preparations were the same for all experiments). 150 single shot spectra were accumulated for each delay time. The distance from the probe surface to the center of the drawout window (defined as the “probe distance”) was 14.5 mm. Plotted is the measured ion density of the protonated peptide molecular ions (m/z 1085) against translational energy of the ions.

Figure 2. Lysozyme/nicotinic acid mixture by UV/LDI. 1 ul 10^-6 M peptide aqueous solution and 1 ul matrix solution were used. 200 single shot spectra were accumulated for each delay time. The probe distance was 2.5 mm. Plotted is the measured ion density of peptide molecular ions (m/z 14300) against translational energy of the ions from two experiments. Ion densities from the experiment using lower laser irradiance were scaled up to give a better comparison of the two experimental results.

Figure 3. Gramicidin by IR/LDI. 1 ul 10^-4 M peptide aqueous solution was used. The laser was operated at 1 Hz. 50 spectra were accumulated for each delay time. The probe distance was 15 mm. Plotted is the measured ion density of the protonated peptide molecular ions (m/z 1198) against translational energy of the ions.

Figure 4. Nicotinic acid by UV/LDI. 1.5 ul 10^-5 M nicotinic acid aqueous solution were used. The Q-switch was operated at 2 Hz. 50 spectra were accumulated for each delay time. The probe distance was 14.5 mm. Plotted is the ion density of the protonated nicotinic acid molecular ions (m/z 124) against translational energy of the ions from three experiments.

References
A UV Laser Desorption Mass Spectrometer for Routine Use in a Life Science Laboratory.

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Following Hillenkamp and Karas' revolutionary announcement of desorption of very large biopolymers using UV laser mass spectrometry (LD), several groups have started building UV-LD mass spectrometers. However, laser desorption mass spectrometry is still operationally complex, mainly owing to the difficulties in finding and reproducing correct measurement parameters and sample preparation conditions. Furthermore, systems used so far have usually been designed for and used in research environments.

Here we describe a UV LD mass spectrometer built with the following goals in mind: (a) ease of use, (b) high sample throughput, (c) low maintenance, and (d) sensitivity, resolution and mass accuracy as high as other published systems. We modified a Bio Ion 10 mass spectrometer originally used for plasma desorption mass spectrometry, which makes sample introduction both convenient and efficient. A low cost and virtually maintenance-free nitrogen laser was coupled via an optical bench to the apparatus. Laser intensity and the position of the laser spot on the target can be adjusted by elements on the optical bench.

A digital oscilloscope (LeCroy 9450) measures the output of a double channeltron with up to 400 megasamples/s and with a memory depth of 50000 samples. The data is transferred to a Macintosh II computer where it can be displayed and manipulated using a program written by us. That program, which also controls the laser, follows standard Macintosh conventions for the graphical user interface making it easy to use. All acquisition parameters are controlled from the computer screen limiting operator interaction with the instrument to sample introduction.

As table 1 shows, the apparatus is capable of excellent mass accuracy, approaching that of electrospray data. In our preliminary experience we find this accuracy to be typical of proteins for which an adequate signal - to - noise ratio can be obtained by LDMS. Results for the resolution (table 2) match published results.

We conclude that the hardware of our simple device is fully competitive with other systems while operation is made easier. In figure 2 one of our first applications of this instrument to the solution of problems in protein chemistry is shown. Further work will focus on improving the signal - to - noise ratio, developing software to automatically perform most of the acquisition process and on sample preparation to make LDMS results more reproducible.

Table 1. Preliminary Results for Mass Accuracy Using A Series of Parvalbumins:

| Species: | Carp | Hake | Pike | Leop. Shark | Thorn. Ray*
|---------|------|------|------|-----------|-----------
| $M_r$ (sequence) | 11478 | 11337 | 11820 | 11932.5 | 11821
| $M_r$ (experimental)** | 11477.2 | 11338.2 | 11819.3 | 11931 | 11836.7*

* the signal to noise ratio was very low in this sample
** masses where determined using insulin and ribonuclease A as internal standards
Table 2. Preliminary Results for Resolution:

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* Resolution as full width at half height (FWHH).

Figure 1. LDMS can be used to rapidly check the correct expressing of recombinant AcylCoA Binding Protein (ACBP) in different host organisms. The top part of the left figure shows a spectrum of recombinant ACBP expressed in E. coli. The bottom part shows recombinant ACBP derivatized for binding studies. The expected mass difference of 104 Da is confirmed by LDMS.

Figure 2 (below). Rapid check of the homogeneity of recombinant ACBP. Comparison of its peak width those of Insulin and Myoglobin shows that this ACBP sample is heterogeneous (here probably due to partial oxidation of the two methionyl residues).
Matrix-assisted laser desorption (MALD) ionization provides a unique opportunity to probe fundamental processes involved in desorption ionization. For instance, there seems to be little doubt that the MALD process is initiated by resonant photon absorption (probably single photon, e.g., S\textsubscript{0} --- S\textsubscript{1}) by a molecule in a dense medium. Although MALD can be achieved by pumping higher lying electronic states of the matrix, there are probably very few advantages to using higher photon energies. In fact, there may be distinct disadvantages for use of higher photon energies. The rational for this view is based on our current view of the MALD process. Figure 1 presents a pictorial view of our proposed mechanism. The steps include: 1) photon absorption to produce an ensemble of S\textsubscript{1}; 2) internal conversion of some S\textsubscript{1} species results in heating of a localized region; 3) the rapid heating causes ejection of "cluster species" from the surface, e.g., [(S\textsubscript{1})\textsubscript{n} --- S\textsubscript{0}] or [(S\textsubscript{1})\textsubscript{n} (A\textsubscript{n}) --- S\textsubscript{0}], where A represents analyte molecules; 4) the enhanced acidity of S\textsubscript{1} (relative to S\textsubscript{0}) results in proton transfer to yield [(S\textsubscript{1})\textsubscript{n} (A\textsubscript{n}) --- AH\textsuperscript{+} --- S\textsubscript{0}]; 5) evaporation of the cluster yields solvated adducts, [(S\textsubscript{1})\textsubscript{n} --- AH\textsuperscript{+}] and [(S\textsubscript{1})\textsubscript{n} --- S\textsubscript{0}] or intact analyte molecular ions [A + H\textsuperscript{+}].

The proton transfer will be quenched if S\textsubscript{1} decays by radiative or non-radiative pathways, e.g., S\textsubscript{0} --- A. Coupling of electronic energy transfer can be eliminated by using matrices where the adiabatic transition energy (E\textsubscript{a}) for the matrix molecules is less than that for the analyte (Figure 2). In general, pumping of high energy transitions, S\textsubscript{1} --- S\textsubscript{2}, will be less effective for MALD because of electronic energy transfer and the coupling of these states to the triplet manifold. For example, the types of molecules that work best for

---

MALD, polar aromatics, the higher lying electronic states are more strongly coupled to the triplet manifold, this will be advantageous only if the acidity of the $T_1$ state is sufficient to promote the proton transfer reaction. Note: There are some cases where the acidity of the triplet is greater than the acidity of the singlet state.

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HPLC-Frit-FAB-MS of Peptides Using Thioglycerol as Matrix

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HPLC has been effectively coupled to mass spectrometry using several continuous-flow (CF)-FAB or LSIMS methods. CF-FAB has also been used in a flow-injection mode because of reports of lower matrix interference and decreased suppression of ion signals in mixtures as compared to static FAB. In most situations, a liquid matrix is added to the LC mobile phase or added co-axially post-column to improve the stability of the secondary ion beam. Glycerol is used almost exclusively for this purpose, although many other matrices (eg. thioglycerol, nitrobenzyl alcohol, etc.) are commonly employed for static FAB (1).

In a preliminary set of experiments, we observed that thioglycolrol rather than glycerol in the mobile phase during LC-MS decreased the matrix background and improved the ion signals from peptides. This study attempts to characterize the utility of thioglycerol in LC-frit-FAB-MS.

When glycerol is used for a matrix in either static or CF-FAB, a high background of clusters is evident. The regular pattern of ions m/z 645, 737, 829, etc correspond to clusters of (glycerol_n + H)^+. Although these ions are suppressed somewhat when sample elutes and can be further reduced by background subtraction, they still pose a potential interference if they coincide with the mass of the molecular ion or important fragment ions. The background from thioglycerol is substantially less, especially at higher mass (ie. > m/z 600).

Figure 1 shows a comparison between the absolute abundances of peptide ions when glycerol and thioglycerol were added as the matrix. The protonated molecular ion and a series of sequence-specific fragment ions were considered. Although the abundance of sequence-related fragment ions is roughly comparable in either glycerol or thioglycerol, the abundance of the protonated molecular ion is substantially higher in thioglycerol than in glycerol. This enhancement is 2 to 4-fold for the peptides tested and, together with the decreased background, leads to an even greater improvement in signal/noise. The effect seems to be exaggerated at higher molecular weight (> 2000).

The optimum conditions for LC-MS using glycerol as a matrix have been reported (2). Because of the lower viscosity and higher volatility of thioglycerol, one might expect optimum conditions to be somewhat different. Fig. 2 shows the sensitivity of peptide ions as a function of thioglycerol content (flow injection). Both the protonated molecular ion and selected fragment ions reached a maximum at about 2.5% thioglycerol. At lower percentages of thioglycerol, the ion signal was somewhat unstable and the peak shape was poor or split. The poor peak shape could be overcome to some extent by increasing the flow rate of the solution from 5 ul/min to 15 ul/min, but the ion source pressure was correspondingly higher.

Ion source temperature was varied from ambient to about 55 ° C. The optimum was 40-42 °, with a sharp drop in sensitivity above 50 °. This is somewhat lower than the optimum for glycerol (50-55 °), as would be expected based on the higher volatility of thioglycerol.

A possible concern of using thioglycerol as a mobile phase additive in LC-MS is that peptides containing disulfide bonds may be reduced by the thiol. To test this, an oxytocin metabolite containing a disulfide was analyzed by static FAB in glycerol both before and after 24 hr in a solution of acetonitrile:water:TFA:thioglycerol (2%). Some slight increase in the amount of reduction was apparent, but the predominant species was still the peptide with an intact disulfide bond. The acidity of the mobile phase used here and in most reversed-phase LC experiments undoubtedly helps to prevent more extensive reduction.
LC-MS using thioglycerol in the mobile phase produced the same improvement in peptide signal that was observed in flow injection. Peak shapes were slightly broader and reproducibility was somewhat poorer than when using glycerol in the mobile phase. Although the LC-MS results when using a 4.6 mm (i.d.) column with post-column stream-splitting showed the same improvement as did flow injection, when 1 mm columns or packed capillaries (350 μm, i.d.) were used, some degradation of the column performance seemed to occur in the presence of thioglycerol. The performance could be recovered by washing extensively with methanol. It is unclear at this time if the poorer performance resulted from some alteration of the interaction between the analyte and the stationary phase, or was simply a general increase in baseline caused by thioglycerol, but further study of this phenomenon is warranted.

REFERENCES


FIG. 1
ION ABUNDANCE IN GLYCEROL AND THIOGLYCEROL

FIG. 2
Ion Abundance vs. Percent Thiglycerol in Mobile Phase
FACTORS AFFECTING BAND BROADENING IN LC-FABMS SYSTEMS USING PRECOLUMN ADDITION OF THE VISCIOUS MATRIX

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In systems using continuous flow fast atom bombardment to interface liquid chromatography to mass spectrometry (LC-FABMS), the addition of a viscous matrix to the mobile phase is essential for the proper ionization of the analytes to occur. Although post-column addition of the matrix has been suggested, it is usually easier and more common to achieve analysis by pre-column mixing of the matrix to the mobile phase. In the latter case as the concentration of the matrix exceeds a few percent the overall chromatographic performance of the system is reduced as shown recently using compounds such as peptides, organic acids and phenolic compounds (1). The broadening resulting from pre-column addition can be examined by monitoring the changes in the normalized peak width (w₁/₂/tn). As shown in Figure 1 the increase in Gly content between 0-30% induces broadening (w₁/₂/tn). For p-hydroxybenzoic acid, the less retained compound, broadening can be as high as 67% at 30% Gly. For vanillic acid and met-enkephalin the increases in broadening are 37% and 28% respectively. This correlates well with the decrease in number of theoretical plates and loss in resolution observed with the increase in Gly content (1). In order to find the source of broadening examination of the variances associated with the system is necessary.

In multi-component systems such as LC-FABMS, induced broadening of the input signal can be described by the sum of individual variances associated with each component. The total variance, σ² = σ²(chr) + σ²(int) + σ²(ms), as shown in equation [1].

σ² = σ²(chr) + σ²(int) + σ²(ms)  [1]

The chromatographic variance (σ²(chr)) can be expressed as the sum of the variance occurring in the column (σ²(c)) and extra column broadening (σ²(ex)). Broadening occurring within the column results from kinetic (σ²(k')) and extra column broadening (σ²(ex)).

H = A exp(B/Gly) + C/Gly + Dm + T (1 + E + 1/k' + 1/Dm)² [2]

σ²(chr) = σ²(chr) + σ²(int) + σ²(ms)  [3]

Figure 2 shows typical Van Deemter plots obtained for met-enkephalin when Gly in the mobile phase was increased from 0-30%. The data indicate that as the concentration of Gly is increased, the C term steadily increases as shown by the increase in the slopes. No significant difference was observed in the C term for Gly values below 3% but as the value increased above 5% the slope steadily increased. Since the C term depends on k' and Dm, it is possible to identify the factors responsible for the loss in efficiency observed in the chromatographic system when the concentration of the matrix is increased.

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In LC-FABMS other variances are also likely to act as dispersion operators. The dispersion operators in the interface are the transfer capillary (0.075 x 1000mm) and the liquid droplet which forms on the probe tip. The total variance (σ²(ms)) associated with the interface is the sum of the variance of the interface and the contribution from the probe tip.
tube ($\sigma^2_d$) and the variance associated with the droplet ($\sigma^2_g$). The contribution of the transfer capillary tubing estimated from the Taylor-Golay relationship is less than 0.15 µL at 30% Gly and this dispersion can be lower for a smaller capillary. Thus the dispersion produced by the capillary is small. Volumetric dispersion also occurs at the end of the probe and it is relatively difficult to evaluate since there are many phenomena occurring in the droplet. These are mainly related to evaporation, mixing and diffusion. In order to estimate that dispersion the droplet can be assumed to act as a "connecting tube" and the variance associated with it can be obtained from the Taylor-Golay relation (3). Alternatively, the droplet can be considered as a mixing chamber in which case the variance associated with it can be obtained from equation [4]. Values for the variance ($\sigma^2_g$) can be obtained (Table 1) using each of these models. The data show that the laminar contribution is superior to the mixing effect. Furthermore, it can be observed that the effect of the addition of glycerol increases the value of the laminar contribution. The dispersion created by the droplet at the tip of the FAB probe is estimated between 0.63 and 1.47 µL when the concentration in glycerol is around 30%. These values appear as minimal since other broadening phenomena are occurring at the tip and it is known that the concentration of Gly on the tip can be as high as 90%. A dispersion of 1.47 µL appears reasonable under those circumstances and the main contribution to broadening of the interface is due to the formation of the liquid droplet which represents ≈35% of the total broadening measured ($1.47^2/2.5^2$). Thus, the measured dispersion implies that the interface can contribute significantly to the broadening of the chromatographic band especially for narrow bands such as those found with capillary columns. The signal can also be broadened by the mass spectrometer ($\sigma^2_{ms}$) if the scanning speed of the instrument is too slow. For compounds that elute in narrow bands, the cycle time should be of the order of 0.5-1 second to maintain chromatographic integrity and longer cycle times should be avoided.

![Graph](image1.png)

**FIGURE 1.** Variation in the normalized peak width ($w_{l/g}/w_t$) with glycerol content. O: Vanillic acid, D: mel-enkophallin, A: p-hydroxybenzoic acid.

![Graph](image2.png)

**FIGURE 2.** Van Deemter plots obtained for mel-enkophallin with glycerol concentration at 0% and 30% Gly.

![Graph](image3.png)

**FIGURE 3.** Variation of the $k'/Dm$ with glycerol concentration. O: Vanillic acid, D: mel-enkophallin, A: p-hydroxybenzoic acid.

**TABLE 1**

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(1) Calculated at 0% glycerol
(2) Calculated at 30% glycerol

**REFERENCES**

1. J.P. Gagné, A. Carrier and M.J. Bertrand, J. Chrom., (in press);

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SENSITIVITY ENHANCEMENT FOR STATIC AND CONTINUOUS FLOW FAB/MS ANALYSIS OF NUCLEOTIDES BY QUATERNARY AMINE SURFACTANTS

Steven M. Musser and James A. Kelley
Laboratory of Medicinal Chemistry, DTP, DCT, NCI, NIH, Bethesda, MD, 20892

Our laboratory is concerned with the in vitro and in vivo analysis of nucleoside analogs and their metabolites as part of the preclinical evaluation and development of these compounds as new anti-AIDS and antitumor agents. These drugs are usually activated by intracellular phosphorylation. Identification of phosphorylated metabolites is therefore important in determining the mechanism of action and assessing the effectiveness of these drugs. Nucleotide metabolites isolated from cells have proven to be difficult to analyze using mass spectrometry because of salt contamination, low sensitivity and chemical instability. Continuous flow fast atom bombardment (CF-FAB) has been shown to improve sensitivity for nucleotides when compared to static FAB (1), however, the sensitivity is not high enough for identification of nucleotide metabolites from biological matrices. SIMS experiments have demonstrated a dramatic increase in sensitivity for adenosine triphosphate when hexadecylpyridinium acetate was with the matrix (2). Therefore, in this study we have evaluated the utility of quaternary amine surfactants for CF-FAB analysis of nucleotides.

A 300-350 µl/min flow from a Spectra-Physics model 8800 ternary gradient HPLC pump was split prior to the injector to provide a flow rate of 4-5 µl/min. A 0.30 mm i.d. X 250 mm glass lined stainless steel capillary column was packed with 3 µm Nucleosil C18 and used for the separations. For CF-FAB experiments the following mobile phases were mixed to provide optimum elution of the various nucleotides: (A) 94 % H₂O:5 % glycerol:1 % ACN, containing 10 mM ammonium formate and 50 µM dodecyl trimethylammonium hydroxide (pH 8.2); (B) 45 % H₂O:5 % glycerol:50 % ACN, containing 100 mM ammonium formate and 50 µM dodecyl trimethylammonium hydroxide (pH 8.2). Mass spectra were obtained on a VG 7070E mass spectrometer (6 kV accelerating voltage) using xenon fast atoms for ionization. A mass range 1100 - 100 amu was scanned at a rate of 10 sec/dec for all experiments. For static FAB experiments, 1 µl aliquots of the nucleotides dissolved in H₂O were mixed with matrix on the probe tip. For CF-FAB experiments 2 µl injections of the nucleotide standards were made onto the µ-HPLC column. The eluent from the µ-HPLC column was directed into the ion source via a 1 m X 50 µm i.d. fused silica capillary. In CF-FAB experiments the probe tip was heated to 55 °C.

Use of the quaternary amine surfactant, dodecyl trimethylammonium hydroxide, along with µ-column HPLC-CF-FAB provides several advantages over CF-FAB and static FAB for nucleotide analysis:

1. The quaternary amine surfactant serves as a an ion pair reagent that facilitates nucleotide separation on reverse phase columns.
2. Use of a column removes the majority of salt interferences from the sample. This increases sensitivity and decreases FAB spectrum complexity.
3. The physical properties of the surfactant increase the "wetability" of the probe tip and decrease the probe tip and decrease the surface tension of the matrix film. This results in better flow stability and less noise.
4. Overall, using the proper surfactant provides a 10X increase in sensitivity over CF-FAB alone and a factor of 100X increase over static FAB analysis.

While some longer chain surfactants provided slightly better sensitivity, dodecyl trimethylammonium hydroxide resulted in better overall performance, particularly in terms of chromatographic separations.


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Figure 1. Relationship between increasing surfactant chain length and sensitivity for nucleotides in static FAB as measured by the (M-H)^- ion of deoxycytidine monophosphate.

Figure 2. (A) Unsubtracted negative ion CF-FAB mass spectrum of 2 ug (4.5 nMoles) of cytidine-diphosphoethanolamine. (B) Unsubtracted negative ion CF-FAB mass spectrum of 100 ng (225 pMoles) of cytidine-diphosphoethanolamine with surfactant in the mobile phase.
Continuous Flow-Fab Mass Spectral Analysis of UDP-Sugars from Mammalian Cells

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Interest in the analysis of UDP-sugars is attributed to their position in cells in the synthesis of a wide array of biochemical processes. The polarity and lack of volatility of these compounds make them ideal candidates for LC-MS.

Detection and identification of UDP-hexoses and UDP-hexosamines in extracts of mammalian cells (mouse leukemia, L1210) was accomplished by ion-pair microcolumn LC-frit FAB MS. The system comprised a 0.25 x 250 mm stainless steel glass lined capillary packed with 3 μm particles (C-18, Nucleosil) interfaced via a 1 m capillary to the FAB source. Mobile phase was split prior to the injection site such that flow into the source was 4-5 μl/min. Components of the mobile phase were 10 mM ammonium formate and 25 μM dodecyl trimethylammonium formate, pH 8.2, with 1% each acetonitrile and glycerol (A) or 100 mM ammonium formate and 25 μM dodecyl trimethylammonium formate, pH 8.2, with 50% acetonitrile and 1% glycerol (B). From 0-10 min, B was increased linearly from 0 to 15%. Under these conditions, UDP-glucose and UDP-xylose elute at 17.0 min, and UDP-N-acetylglucosamine at 20.5 min. The peaks overlap, but their maxima are resolved enough to allow for clean spectra.

Cell extracts were prepared from cultures grown in suspension by precipitating rinsed cells in a 60% methanol. Dried extract residue was filtered once, subjected to preparative HPLC, and the eluate refiltered prior to LC-MS analysis. The preparative LC step was necessary to isolate the UDP-sugar fraction from other nucleotides and salts which could interfere in the analysis and clog the microcolumn. Despite this precaution, there was evidence of salt suppression of ionization of cellular UDP-sugars.

A standard FAB spectrum of UDP-glucose shows a M-1 ion at 565 amu, as well as an ion for glucose-1-diphosphate (339 amu). UDP-N-acetylglucosamine yielded a M-1 ion at 606 amu and fragments at m/z 300 (N-acetylglucosamine-1-diphosphate) and m/z 300 (N-acetylglucosamine-1-phosphate). A mass spectrum of L1210 cell extract contained ions 565 and 606, but not the M-1 for UDP-xylose. In addition to the M-1 peak, a UDP-xylose standard spectrum had peaks for fragments xylose-1-diphosphate (309), xylose-1-diphosphate - H₂O (291), and xylose-1-phosphate - H₂O. All three UDP-sugars fragmented to UDP (m/z 403) and uracil (m/z 111).

The presence of UDP-glucose and UDP-N-acetylglucosamine in L1210 cells was confirmed by HPLC using a variable wavelength detector monitoring 262 nm.

1Personal communication, Cooney and Christine Chisena, NCI.
Negative ion mass spectrum of the peak at 17.0 min. in the HPLC chromatogram of the cell extract.

Frit-FAB negative ion mass spectrum of UDP-glucose standard.
Analysis of cloricromene acid levels in the rabbit plasma by continuous-flow fast atom bombardment (CF-FAB) mass spectrometry

S. Chen, L. Zangirolami, R. Mariot, P. Bellato, G. Menon and *P. Traldi
FIDIA Research Laboratories, 35031 Abano Terme, Italy and *CNR Mass Spectrometry Service, 35200 Padua, Italy

Cloricromene acid is a stable catabolite of cloricromene that inhibits the platelet aggregation (1-2), known to be formed through the hydrolysis of the ester bond within this coumarin derivative (Fig.1 and 2) in plasma (3). The measurement of the acid levels in plasma is essential for an understanding of disposition of cloricromene in vivo. We use CF-FAB to quantify the acid in the rabbit plasma after an intraduodenal administration of cloricromene. Work was done on a VG-ZAB-2F mass spectrometer with its standard ion source and CF-FAB probe with a one-meter-long 75 um capillary column using methanol:water:thioglycerol (70:27:3) as the mobile phase at a flow rate of 3-5 ul/min provided by a micropump. Calibration curve was prepared by combining various concentrations of the acid with constant amount of the internal standard (5 ng of cloricromene). Quantitative range for the acid is 1-25 ng plotted by the measurement of the peak area ratios (Fig.3). A summary of the procedures for analysis of the acid is shown in Fig.4. Fig.5 shows the decay curve of cloricromene acid in the plasma from 3 rabbits after the administration. For evaluation of the results obtained by CF-FAB method we also quantified the acid levels in the rabbit plasma by reverse phase high performance liquid chromatography combined with fluorescence detector by measurement of the peak area of the acid. The data obtained by the two methods present a good agreement.

References

2) M. Prosdocimi et al. N-S Arch Pharmacol. 332; 305 (1986).
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

**Fig. 1**
- Clorlcromene acid
- Biological samples

**Fig. 2**
- Clorlcromene acid
- MW 367.8

**Fig. 3**
- Plot of Clorlcromene acid (ng)
- Equation: $Y = 0.037X + 0.073$
- $r = 0.997$

**Fig. 4**
- HPLC analysis
  - Column: C18
  - MP: pH 5.0/Phosphate buffer 5% (25 mM) 3.5% (25 mM) 1% (25 mM)
  - Flow: 3.5 mm/min

**Fig. 5**
- Graph showing

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Analysis of the molecular species of phospholipids from fish liver by continuous-flow fast atom bombardment (CF-FAB) mass spectrometry

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Mass Spectrometry Service, 35200 Padua, Italy

Mass spectrometry has grown increasingly useful for characterizing phospholipids, and fast atom bombardment ionization is currently the most widely used mass spectrometric method for analysis of intact molecules of the species from biological samples because of its some advantages, such as capability of directly analyzing the species from crude extract. Despite its achievement, static FAB has several drawbacks. Ion suppression effect that often occurred with mixture analysis of the compounds hampered to obtain evidence for identification of the fatty acid composition in the species. We used CF-FAB to identify natural phospholipid species from biological sample. The total lipids were extracted from 50 mg liver tissue from an alive torta fish by the method of Bligh and Dyer, and then choline phospholipids were isolated from others on a silica acid column. CF-FAB was carried out on a VG ZAB-2F with its standard source, gun and CF-FAB probe with methanol:water:thioglycerol (70:25:5) for positive ion analysis and methanol:water:triethanolamine (70:28:2) for negative ion measurement as the mobile phase carried by a one-meter-long fused silica capillary column (100 um,i.d.) at a flow rate of 3-5 ul/min. Fig.1 shows the positive and negative ion spectra of choline phospholipids in the fish liver. The molecular species could be identified based on the abundant protonated ions (Fig.1a) and the fatty acid fragments (Fig.1b).

Fig.2 shows the negative ion spectrum of the other classes in the liver. They are mainly phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine. Most of the fatty acid chains containing the classes could be observed from the mixture. Identified molecular species in the liver phospholipids are listed in Table 1. Fig. 3 shows the negative ion spectrum of a crude extract. The main species in the classes could be identified. We used static FAB to analyze the extracts as well. But the unsatisfactory results were obtained.

In summary, CF-FAB method is effective for analysis of phospholipid species because of its major advantage of less ion suppression effect that aids to improve the intensities of useful ions from a mixture. It is also suggested that identification of phospholipid species would become easy if the samples analyzed could undergo a simple isolation as mentioned above before CF-FAB measurement. Influence from the molecular weight overlapping (for example, an ion relates to the two species molecules) would be avoided. The present results also indicated that a high content of 22:6 fatty acid-containing molecular species within major classes of glycerophospholipid is present in this biological sample.
Table 1. Identified the molecular species of the fish liver phospholipids

<table>
<thead>
<tr>
<th>Quasimolecular Ions and Fragments</th>
<th>Identified the molecular species</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMTD (M-16)' (M-81)' (M-67)' (M-COR)' (OCOR)'</td>
<td>PC 16:0-22:6</td>
</tr>
<tr>
<td>834 (PC 40:8) 773 773 747 624</td>
<td>253, 327</td>
</tr>
<tr>
<td>806 (PC 38:8) 781 745 719 498</td>
<td>255, 327</td>
</tr>
<tr>
<td>804 (PC 38:7) 789 743 494</td>
<td>253, 327</td>
</tr>
<tr>
<td>780 (PC 38:6) 789 743 494</td>
<td>253, 327</td>
</tr>
<tr>
<td>760 (PC 34:1) 255, 301</td>
<td>PC 16:0-22:6</td>
</tr>
<tr>
<td>732 (PC 32:1) 255, 253</td>
<td>PC 16:0-18:1</td>
</tr>
<tr>
<td>834 (M-H)'</td>
<td>253, 327</td>
</tr>
<tr>
<td>868 (PI 38:4) 283, 327</td>
<td>PE 18:0-20:2</td>
</tr>
<tr>
<td>858 (PI 36:4) 255, 327</td>
<td>PE 18:0-20:4</td>
</tr>
<tr>
<td>824 (PS 40:6) 253, 327</td>
<td>PE 18:0-20:2</td>
</tr>
<tr>
<td>762 (PE 38:6) 255, 327</td>
<td>PE 18:0-20:2</td>
</tr>
<tr>
<td>738 (PE 36:4) 253, 303</td>
<td>PE 18:0-20:4</td>
</tr>
</tbody>
</table>
CONTINUOUS-FLOW LIQUID SECONDARY ION MASS SPECTROMETRY OF FLAVONOID CONJUGATES

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Flavonoid conjugates encompass a large category of secondary plant metabolites. These compounds are predominantly isolated as O- or C-glycosidic conjugates from complex mixtures and serve a multitude of functions in plant tissues. Recently the isoflavonoid conjugate medicarpin-3'-O-glucoside-6'-O-malonate (MGM) has been shown to inhibit microbial infection in leguminous plant cells. Our primary interest lies in identification and characterization of flavonoid conjugates associated with alfalfa cell cultures.

Owing to the polarity and thermally labile nature of the glycosidic linkage, flavonoid conjugates are not amenable to normal electron impact ionization and GC/MS without extensive derivatization. Low intensity or nonexistent molecular ions of the nonderivatized components are observed in EI spectra. Molecular ion intensities are drastically increased when a "softer" ionization technique such as liquid secondary ion mass spectrometry (LSIMS) is used. Separation of mixtures of flavonoid conjugates can be performed by liquid chromatography. Our goal was to develop a comprehensive method for the separation and characterization of flavonoids. We anticipated that continuous-flow LSIMS (CF-LSIMS) would provide a uniquely beneficial online separation and analysis strategy that would overcome the obstacles previously encountered with GC/MS.

To establish the CF-LSIMS method on the VG ZAB2-SE, a series of flavonoid conjugates consisting of rutin, naringin, and esculin were used. Flow-injection CF-LSIMS was performed with a slightly modified VG continuous-flow probe. Total and selected ion chromatograms for three repetitive injections of rutin and a representative spectrum are given in FIG. 1. Detection limits were then determined for the standards utilizing normal LSIMS and then CF-LSIMS operating in a flow-injection mode. The minimum detectable quantities (MDQ) found for both techniques are presented in TABLE 1. It was found that the detection limits for the CF-LSIMS system were lower than the those of the LSIMS system, which was expected.

A microbore HPLC system was then used to separate and identify a mixture of the above standards. Resulting spectra were similar to that given in FIG. 1. Detection limits were then determined for the mixture through a microbore HPLC interface and the results are also reported in TABLE 1. The results presented elevated detection limits which were attributed to band spreading while on the column and dead volumes associated with the microbore HPLC system.

To remedy any possible dead volumes, preliminary investigation into packed fused silica capillary columns (PFSC) was performed. A UV trace for the separation utilizing a 31cm x 320μm I.D. PFSC is presented in FIG. 2. Spectra obtained were also similar to FIG. 1.

Tandem mass spectra were then acquired for the individual standards by normal LSIMS since future goals will focus on LC/CF-LSIMS tandem mass spectrometry. These spectra correspond to those found in the literature. Tandem mass spectrometry will be used to determine unknown flavonoid conjugates utilizing characteristic fragmentation patterns observed in standards conjugates.

FIG. 1- Total and selected ion chromatograms of three repetitive injections of rutin with [M+H]+= 611.

**TABLE 1-** Minimum detectable quantities (M.D.Q.) of flavonoid standards determined by three separate techniques.

<table>
<thead>
<tr>
<th>Flavonoid</th>
<th>LSIMS</th>
<th>CF-LSIMS</th>
<th>LC/CF-LSIMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NARINGIN</td>
<td>0.5nmol</td>
<td>0.2nmol</td>
<td>2.0nmol</td>
</tr>
<tr>
<td>ESCULIN</td>
<td>0.3nmol</td>
<td>0.3nmol</td>
<td>3.0nmol</td>
</tr>
<tr>
<td>RUTIN</td>
<td>1.6nmol</td>
<td>0.2nmol</td>
<td>3.0nmol</td>
</tr>
</tbody>
</table>

**FIG. 2-** UV chromatogram illustrating the separation of the standard mixture using a 31cm x 320um I.D. packed fused silica capillary column.

M.P. = 60/40 water/methanol w/ 2% gly & 0.1% TFA
DETECTION OF BENZO(A)PYRENE METABOLITES BY CONTINUOUS-FLOW FAST ATOM BOMBARDMENT MASS SPECTROMETRY

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Benzo(a)pyrene (BP) is a common contaminant of the environment. BP has been shown to cause cancer in animals after metabolic activation. The majority of BP is metabolized to water-soluble conjugates of glucuronic acid, glutathione or sulfates. The detection of these conjugates is important since they represent the extent of exposure of the organism to the parent hydrocarbon. Recently, we have reported the development of a continuous-flow FABMS method for the detection of BP-sulfates in cell cultures. In this paper, we present the results of the application of a similar method for the detection of BP-glucuronides from cell media.

Media from BP-treated cell cultures of hamster embryo cells, mouse hepatoma cell line Hepa-1c1c7, and the human hepatoma cell line HepG2 were extracted with ethylacetate/water after adjustment of pH to 4.5. The ethylacetate portion was fractionated by reversed phase HPLC (1). Fractions corresponding to the retention time of authentic BP-glucuronide were analyzed by continuous-flow FABMS (1). On-line experiments were performed using a 1 mm ID microbore column.

A resolution of 10,000 was necessary to separate molecular anion from background signal. Although this caused attenuation of the signal by ten times, a detection limit of a picogram was achieved. Fractions from HPLC analysis of media from BP-treated and untreated Hepa-1c1c7 cells (Fig. 1a and d, upper) were analyzed by continuous-flow FABMS. Single ion recording of fractions from BP-treated Hepa-1c1c7 cells gave response (Fig. 1a, lower) while fractions from untreated cells gave no response (Fig. 1d, lower). The same results were observed for hamster embryo cells. No response was observed for BP-treated HepG2 cells. Calculations based on standard curve and recovery studies indicated that the concentration of BP-glucuronide in the media of BP-treated Hepa-1c1c7 and hamster embryo cells was 250 ng/ml and 90 ng/ml, respectively. These indicated that Hepa-1c1c7 cells and hamster embryo cells have converted 18 % and 25 % of the BP into BP-glucuronide, respectively. The minimum amount that could be quantitated by this method was 0.5 ng. This suggested that HepG2 cell culture, which showed no signal, have converted less than 0.05% of the BP into BP-glucuronide. When BP-treated cells were analyzed by on-line HPLCMS (under conditions different than used in off-line HPLC fractionation), two partially resolved peaks were observed (Fig. 2a and b). On-line LCMS analysis of untreated cells (Fig. 2c) and a mixture of authentic samples (Fig. 2d) indicated that these partially resolved peaks were isomeric phenolic glucuronides.

These results demonstrate that continuous-flow FABMS can be used to analyze BP-metabolites in cell cultures. The sensitivity and detection limit are with in the range at which biological studies are carried out. The method can also be used for the analyses of other hydrocarbons. The on-line experiments demonstrate a rapid and simple method of detection and identification of metabolites.

REFERENCES

Figure 1. UV and continuous-flow FABMS analysis of BP-glucuronide from the medium of BP-treated Hepa-1c1c7 cells. Upper: UV-recordings of HPLC fractions of medium from cells treated with BP (a), and untreated (d). Arrows indicate the time at which fractions were collected and analyzed. Lower: Single ion recordings for (a) medium from cells treated with BP, (b) 300 pg of authentic BP-glucuronide, (c) solvent blank and (d) medium from untreated cells. Arrows indicate time of injection.

Figure 2. Single ion recordings (m/z 443) for on-line LCMS analysis of: a) BP-treated Hepa-1c1c7 cells, b) BP-treated hamster embryo cells, c) Hepa-1c1c7 cells not treated with BP, and d) a mixture of authentic 1-BP-glucuronide and 3-BP-glucuronide.
FLOW FAB/MS, MS/MS, AND MICROBORE LC FLOW FAB/MS OF CYCLOATE METABOLITES

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Cycloate is a selective, pre-plant incorporated, thiocarbamate herbicide used primarily in sugar beets. In a crop rotation study the metabolism of cycloate was studied in radish and spinach by flow FAB/MS, MS/MS, and microbore LC flow FAB/MS.

The CAD spectra of a series of suspected metabolites were obtained by flow FAB MS/MS on a Finnigan MAT TSQ 70. Good quality spectra were obtained at 5-50 ng injections. Methanol extracts of leaves from plants grown in cycloate treated soil were also examined by flow FAB MS/MS. Prior chromatographic data suggested that N-ethyl-N-cyclohexylamine and the N-malonylcysteine conjugate of cycloate were present in some of the samples. CAD spectra of the extracts confirmed the presence of only N-ethyl-N-cyclohexylamine. An extract was then partially purified by preparative TLC and the band that co-migrated with the N-malonylcysteine conjugate of cycloate was re-examined by flow FAB MS/MS. The resulting spectrum verified the presence of the conjugate.

Microbore LC flow FAB was done to identify unknown metabolites. This was accomplished with a 0.322 mm diameter C-18 microbore LC column (LC Packings) that was 130 mm in length. An unknown cycloate metabolite which had been partially purified by HPLC was chromatographed on the microbore LC column using .1/10/30/60 trifluoroacetic acid/glycerol/acetonitrile/water. The spectrum obtained (top Figure) is consistent with a malonyl derivative of a cycloate glycoside. Flow FAB MS/MS (bottom Figure) located the cyclohexyl ring as the site of cycloate hydroxylation.
Alkyl Sulfate and Sulfonate Analysis Using Negative Ion Continuous Flow FAB LC/MS on a Magnetic Sector Instrument
J. G. Nowlin, J. Gumulka, H. N. Diep, I. Dzidic
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Introduction:
A reliable analysis for alkyl sulfates and sulfonates capable of providing both qualitative and quantitative data has been of interest to the surfactant industry for some time. Within the last two years a reverse phase ion-paired liquid chromatographic separation of alkyl sulfates and sulfonates using conductivity detection was implemented at Shell’s Westhollow Research Center. The same LC method has recently been interfaced to Continuous Flow Fast Ion Bombardment on the VG TS 250 mass spectrometer to determine whether or not the negative ion CFFIB analysis of 5 μl (1%) of the HPLC affluent would produce adequate signals for identification of the sulfates and sulfonates.

Experimental:
Reference mixtures containing 5 to 50 ppm of alkyl sulfates and sulfonates were used for the LC-CFFIB evaluation. Three percent glycerol was added to the mobile phase and the LC system re-evaluated for chromatographic effects. The methanol/water gradient was modified to optimize the separation and to compensate for peak broadening caused by the addition of glycerol. The HPLC gradient HPLC program included:

A: 40% MeOH + 3% Glycerol + 10%(0.4g/L)NH₄Ac in H₂O
B: 80% MeOH + 3% Glycerol + 10%(0.4g/L)NH₄Ac in H₂O

Program
0 - 9 minutes: A=65% B=35%
10 - 15 minutes: A=55% B=45%
16 - 30 minutes: A:65% B=35%

The C₈ analytical column (Custom LC, 15cm x 4.6mm) used for the separation was maintained at 40°C to minimize back pressure caused by the addition of glycerol.

Fifty μl of the 5 to 50 ppm test samples was injected onto the C₈ column with a 0.5 ml/min flow rate. The flow was split 100:1 in such a way that the 1% or 5 μl was directed to the mass spectrometer and the larger portion of the flow was directed to the conductivity detector for additional monitoring of the separation.

The TS 250 FIB source was heated to 120°C for the flow FIB experiments. Under these conditions, the system produced a stable beam and was reliable and reproducible over several days of testing.

Sensitivity:
Sample concentrations delivered to the mass spectrometer ranged from 12.5 ng in the hexadecene sulfonate mixture to 0.5 ng of individual disulfonates in the alpha olefin blend. This series of experiments illustrates that the LC-FIB system is capable of detecting sulfates and sulfonates at concentration levels below 1 ppm.

Conclusion:
Existing analytical LC methods using gradient elution can be easily interfaced to CFFIB. The 1% of the total flow directed to the mass spectrometer produced adequate negative ion responses to allow identification of the alkyl sulfates and sulfonates. Even with the 100:1 split, these test samples overloaded the system. The most desirable peak shape and quality spectra were observed on the impurities not previously detected by LC alone. Sensitivity of the method was very satisfactory.
Acknowledgements:

We would like to express our appreciation to Vic Parr and Dave Wood (SAL, Manchester, UK) for their assistance in establishing the CFFIB analysis and for their support of the TS 250.

We would also like to acknowledge the support of the VG companies with special thanks to Rahil Amin, Scott Campbell, Dan Carlile, Ian Groove, Max Letcher and Roberto Raso.
ELECTROACCUMULATION OF SAMPLES FROM ELECTROPHORETIC GELS FOR MASS SPECTROMETRIC ANALYSIS

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School of Chemistry & Biochemistry
Georgia Institute of Technology, Atlanta, GA 30332

At the 1990 ASMS, we introduced a procedure that used mechanical disruption of an isolated area of a planar electrophoretic gel using either a mechanical rotor/stator or piezoelectric homogenizer. These homogenizers, used in combination with chemical degradation of the gel, created a microslurry of gel particles and sample molecules dispersed in an aqueous extraction solvent. A small amount of this suspension, ranging in volume between 50 and 1000 microliters was processed through a particulate filter and a concentration column. This processing allowed a sharp plug of fairly concentrated sample to be passed through a capillary transfer line to a flow FAB ionization source on the mass spectrometer. This device was successful in reaching the time goal and the extraction efficiency, and was demonstrated for samples such as small peptides, angiotensin, and vitamin B12.

A separate presentation at this meeting summarizes work that we have completed using direct laser desorption with matrix assistance for the determination of samples using a Fourier transform ion cyclotron resonance mass spectrometer. Some success is evident, but several other methods are more generally successful in preparing the sample. These include the “freeze-squeeze” method of releasing sample molecules from within agarose gels, and electroelution into small quantities of buffer solution deposited on the top of an aqueous gel. Recoveries for these methods have been determined to be high, ranging up to 80% for some samples. A few ng (a few pmoles) of sample applied to the electrophoretic gel was sufficient to generate useful mass spectra, and in some cases, collision-induced dissociation MS/MS spectra. The three sections of this presentation focus on electroelution onto nitrocellulose membranes and collection electrodes, electroaccumulation onto an optically transparent surface so that UV/vis or fluorescence spectroscopy might be used to monitor the accumulation of sample, and the release of samples from modified gels, especially low melting point agarose gels.

ELECTROELUTION: The initial device constructed for these experiments consists of two metal electrodes. A platinum circle or stainless steel tube is used to isolate the sample band on the electrophoretic gel. A metal wire electrode is placed in the center of the circle; it is on this wire that the sample accumulates. The potential applied to the metal wire electrode is opposite of the polarity of the sample molecule in the buffer solution used or added to the local area. The electrophoretic band can be moved rapidly to the central electrode under the influence of the applied potential, limited only by the ability of the gel itself to support the gradient. The relatively small area of the wire electrode means that only a small amount of sample can be accumulated onto the wire, although the spatial resolution on the surface can be very fine.

Another device used for electroaccumulation based on some modifications of standard technology. Two electrodes are used in conjunction with a small nitrocellulose membrane. The platinum wires are positioned in such a way that the sample band and the nitrocellulose are adjacent and within the potential gradient. Again the potential of the electrode is set to move the charged sample ions towards the nitrocellulose. Up to 50V can be applied to the system for short periods of time, using a nitrocellulose membrane with a pore size of 100 microns. Recovery time is presently in the range of 2 to 3 minutes. A standard extraction procedure is used to remove SDS and stain molecules (if present). This involves a Konigsberg acetone precipitation and occasionally a short centrifugation. The sample can be extracted from the nitrocellulose with methanol. The nitrocellulose must be chosen with care. Most commercial nitrocellulose membranes sold for the purposes of electrotransfer include 3% by weight of a wetting agent, which is a surfactant polymer. This fact is seldom explicitly noted on the specifications sheet. The surfactant gives a high quality FAB mass spectrum and swamps out signal from the sample.

ELECTROELUTION ONTO OPTICAL SURFACES: Electroelution accomplished on a rapid time scale has to be accompanied by some indicator that signals when the sample has been transferred or
accumulated to some predetermined level. Collection of the sample onto nitrocellulose generally proceeds to completion, and ample time is allowed for that situation to be reached. We have used a quartz plate coated with Indium doped tin oxide (ITO) to accomplish electroaccumulation of samples (Figure 1). The ITO coating creates a stable conductive coating on one side of the quartz plate. The ITO plate (a pale brown) can be analyzed during electroaccumulation by UV/vis spectroscopy since it is optically transparent in the range of 220 nm to 1000 nm. UV detection at 254 nm can be used as an indication of the amount of sample on the plate. We also plan to use a fluorescence detector. At present, Coomassie blue staining confirms that protein molecules do move rapidly to the quartz ITO plate. The ITO surface coating is known from independent work to be particularly good for binding of biomolecules through hydrogen bonding. A significant limitation in the present setup is that 5.5 V is the highest potential that can be applied to the sputter-coated plate without loss of the coating. The transfer process must therefore be performed under current controlled and potential controlled conditions (a standard potentiostat is used). The distance between the two electrodes has to be significantly reduced so that the sample recovery time is not excessively long.

**MODIFIED AGAROSE GELS:** Agarose gels can be processed with the "freeze squeeze" method in which the gel is frozen and pressure is then applied to produce a few microliters of solution containing the analyte molecules. Sample recoveries are excellent, but we also wanted to find alternative methods. Agarose gels are available in several modified forms. We performed some initial experiments on a low melting point (about 65°C) agarose gel available commercially. Figure 2 illustrates the sequence of events that releases sample molecules from within the gel within only a few seconds. The gel is cored with a large syringe that has been pre-loaded with a hot 5M KI solution, and for which the syringe has been preheated. The gel cores easily; the potassium iodide prevents resolidification of the gel within the few seconds it then takes to force the resultant solution through a low molecular weight cut off filter, or alternatively a C18 SEPPAK type cartridge. The gel solution passes through the column or cutoff filter, and the sample molecules are held as a plug on the syringe side of the filter or the syringe side of the C18 column. Reversing the flow through the filter passes a concentrated sample through the transfer line to the mass spectrometer, echoing the exact procedure that we described in our previous work.
The direct analysis of substances in liquid matrices by FAB-MS/LSIMS is a powerful addition to the "tool collection" of the mass spectrometrist. In particular, the ability to continuously flow a sample containing solution into the mass spectrometer source substantially increases the types of samples amenable to mass analysis. The main problems in interfacing the liquid and vacuum "phases" are matching the heat flow between the probe surface and the liquid phase to be analyzed prior to vaporization, the creation of a stable liquid phase on the surface of the probe and the requirement that the flow of liquid, as well as gas from a FAB gun, not overload the spectrometers vacuum pumps.

Development of such a device has been in progress in our laboratory in order to bring this capability to a number of quadrupole mass spectrometers, including the HP 598x series. For our HP5982, we have built and begun testing of a very small Continuous Flow-FAB/LSIMS probe which fits through the DIP inlet and utilized a Cesium-ion gun as the primary ion beam. The Cs-gun features a focusable and steerable beam and has the advantage of significantly reducing the gas load on the pumping system. This should also improve the sensitivity since lower source pressures and longer mean free paths should allow better ion collection compared to gas type FAB guns.

Development of such a probe faces severe design constraints: The most demanding aspect is the small size (0.120" dia x .35" long) that such a probe must be to fit this particular ionization source. Other significant problems include the restrictions on primary and secondary ion beam geometries and that of limited primary beam access to the probe tip. We have overcome these problems with a probe design that incorporates both a thermocouple and a nichrome heater within the limited volume available in addition to a fused silica capillary which delivers the solution to the FAB zone. The liquid can flow directly onto the analysis zone or a piece of absorbent material, used as a liquid support. The probe tip and overall experimental setup is shown schematically in Fig. 1.

This probe has thus far been tested on a HP5982 mass spectrometer upgraded with a High Energy Dynode (HED) detector (Phrasor Scientific) and a recently released Cesium Ion Gun (Phrasor Scientific). The probe was tested with a wide range of liquid flow rates (1-20 µL/min using 10% glycerol:water) and demonstrates good sensitivity and almost no memory effects. This is illustrated in Fig. 2 for repeated 0.2µL injections of a 5.4mM sample of dimethyl-dioctadecyl ammonium chloride (Aerosurf, 638 ng/injection) for operation in the selected ion mode ((M-C1)+-551 amu). The probe is also capable of providing high quality mass spectra. This is shown in Fig. 3 for the same Aerosurf solution at a flow rate of 1 µL/min while using the absorbent material. It is important to note that the high mass detection is significantly improved by the use of the HED detector. Work is currently in progress to increase the allowable flow rates and to operate the probe with a variety of solutions.

Acknowledgements
We would like to thank Terry D. Lee and Peter J. Todd for helpful discussions.

References
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Fig. 1 Schematic diagram of experimental setup showing expanded view of probe tip.

Fig. 2 SIM (551 amu) mode plot showing multiple injections of 620 ng Aerosurf. "I" marks time of injection. Flow rate is 8 µL/min of H₂O/glycerol (9:1).

Fig. 3 Aerosurf spectrum. Flow rate of 1 µL/min using absorbent media as liquid support.
CONTINUOUS-FLOW FAST ATOM BOMBARDMENT ON AN EXTERNAL ION SOURCE FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETER

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A continuous-flow (CF) probe interface for fast-atom bombardment (FAB) and secondary ion mass spectrometry (SIMS) has been developed for an external-ion-source Fourier transform ion cyclotron resonance (EIS-FTICR) mass spectrometer. The new interface provides quick and convenient sample introduction and reduces the chemical background associated with the use of a liquid matrix. Medium-resolution broadband, high-resolution heterodyne, and tandem mass spectrometry spectra were obtained on selected peptide samples. The CF-probe interface has also been used to interface a HPLC unit to an EIS-FTICR mass spectrometer.

Since its introduction by Barber, et al. (1) in 1981, fast-atom bombardment (FAB) has become a commonly used desorption ionization techniques for nonvolatile organic molecules. The key benefit of FAB has been the soft ionization nature of the technique that often produces an abundant (quasi-) molecular ion signal. In conventional or static FAB, the sample consists of an analyte dissolved in a suitable liquid matrix. The sample is introduced into the mass spectrometer on the tip of a direct insertion probe (DIP), and is bombarded by a beam of fast (8-10 keV) argon or xenon atoms. While static FAB is a very powerful method, it has some limitations: the analyte signal changes in intensity over time as the matrix evaporates or as the analyte on the target spot becomes depleted, and the liquid matrix produces an abundant background of low m/z ions.

A somewhat new variation of FAB, commonly called continuous flow FAB (CF-FAB) (2), resolves several of these problems and provides additional advantages. The continuous introduction of sample maintains a constant analyte/matrix concentration producing ion intensities that are more stable. This facilitates the optimization of the experimental conditions and provides for increased sensitivity (3). The CF technique provides flexibility in choice of the matrix and often water containing 5 to 10% of glycerol has been used. The lower glycerol concentration reduces the amount of low mass chemical noise (4). The CF-FAB technique also provides a simple interface for liquid chromatography (5).

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The CF-probe interface, illustrated in Figure 1, consisted of a fused silica (0.120 to 0.050 mm i.d.) capillary mounted inside a temperature programmable insertion probe. The heated CF-probe containing the capillary inserts into the external ion source of a Spectrospin CMS-47X mass spectrometer through a standard vacuum port. Liquid samples are introduced directly into the external ion source via a microliter syringe pump. The EIS-FTICR mass spectrometer was equipped with two probe ports located on opposite sides of the external ion source vacuum chamber. Either an Ion Tech FAB gun (10 kV) or a Phrasor Scientific SIMS gun (15 kV) was mounted in the second port opposite the CF-probe. Ions produced in the external ion source were detected with a FTICR mass analyzer. Under CF-FAB conditions, the EIS pressure varied from 4 to 9 x 10^{-4} mbar. The pressure in the FT-ICR mass analyzer high-vacuum chamber was maintained between 5 x 10^{-10} to 3 x 10^{-9} mbar.

The CF-probe interface was used to couple a Bruker LC 21 solvent delivery system for gradient HPLC experiments. A standard reverse phase analytical C18 column 4.6 mm i.d. x 25 cm was used for the HPLC studies. The HPLC solvents were composed of water (94.9% H2O, 5% glycerol, 0.1% TFA) and acetonitrile (60% ACN, 34.9% H2O, 0.1% TFA). The HPLC was operated at a flow rate of 0.5 ml/min. and a split of 500:1 divided the flow between a standard 254 nm UV detector and the CF-probe.

For comparison, a static FAB spectrum obtained from analyzing a 1 μg sample of Gramicidin S in glycerol and a CF-FAB mass spectrum that was obtained on a 500 ng sample of Gramicidin S in a methanol/glycerol matrix are shown in Figure 2. The reduction of glycerol ions (m/z 185, 277, 369...) in the CF-FAB spectrum resulted from the decreased concentration of glycerol in the matrix. Even though more sample was consumed in the static FAB experiment, the measured CF-FAB signal-to-noise ratio of the protonated molecule at m/z 1141.7 was 25 times larger than for static FAB.

Analysis of higher mass ions (m/z > 2000) has been facilitated with the use of the CF-probe interface. A high resolution mass spectrum of melittin from honey bee venom (m/z 2848) in a NBA matrix is shown in Figure 3. The transient signal from melittin lasted over 1 s and a mass resolution of 19,000 (FWHH) was obtained at m/z 2848.
The CF-probe has been used as an interface between a standard HPLC system and an EIS-FTICR mass spectrometer. The 0.5 ml/min flow of effluent from the HPLC was divided between a standard UV detector and the CF-probe. About 2 μg of the total material injected on the column is deposited on the CF-probe tip during the HPLC run. The UV trace recorded for a mixture of the tripeptide, Gly-Leu-Tyr, and angiotensin II is shown in Figure 4. Mass spectra recorded at the maximum ion signal of each HPLC peak are also shown in Figure 4.

The ability to introduce liquid samples directly into an EIS-FTICR has many potential benefits, including stable long-lived signal intensities, a simple liquid chromatography interface, and rapid sample introduction with the potential of high throughput and automation. Coupled with an EIS-FTICR mass spectrometer, the CF-interface provides high mass resolution, high mass accuracy, and MS^n capabilities. Future experiments are planned to evaluate the CF-probe interface with microbore HPLC and capillary electrophoresis.

One of the objectives of our laboratory is to evaluate the utility of tandem mass spectrometry, interfaced with various sample introduction/ionization techniques, in elucidating drug metabolite structures without the use of radioisotopes. For the analysis of drug metabolites and polar drug conjugates, continuous flow-fast atom bombardment (CF-FAB) offers several operational advantages over static FAB. First, the interface allows for direct injection of aqueous samples without removing the FAB target for reloading, thereby avoiding the need to realign the target with the primary ion beam. Second, CF-FAB offers a quantitative comparison of sample injections. Third, because of the decrease in the intensity of matrix cluster ions, a higher relative sensitivity is achieved due to an improvement in the signal to background ratio.

A tandem quadrupole mass spectrometer (Finnigan MAT, TSQ-70) was initially fitted with a commercially available CF-FAB interface (Finnigan MAT, Bioprobe). This interface was difficult to use because it had three centers of alignment: the target, capillary inlet, and FAB gun. It also suffered from severe "memory" effects from previous injections.

A newly designed direct introduction type of CF-FAB probe¹ for a Finnigan TSQ-70 mass spectrometer, similar to that described by Caprioli et al.², was devised to eliminate the problems experienced with the commercial interface. The major design differences were that the inlet capillary line was threaded through a heated direct insertion probe and positioned to extend just beyond the end of a removable target. A filter pad was then used as a "wick", which was mounted to the source housing and positioned to make contact to the underside of the target.

The matrix for CF-FAB was composed of 10% glycerol in 50:50 water/methanol, adjusted to a pH of approximately 3.0 using 1 N HCl. Using a syringe pump (ISCO, model LC-2600), the matrix was delivered through the inlet capillary at approximately 10 μl/min, with an indicated pressure of 50 psi. The CF-FAB probe tip was heated to 30°C. Sample introduction was made manually using a 1 μl loop injector (Rheodyne, model 7410). FAB mass spectra were obtained using a saddle field-type discharge source (Ion Tech, Ltd., model FAB11NF) mounted at a right angle to the CF-FAB probe. Xenon was used as the primary ionizing beam at an energy of 8 keV and an indicated gun current of 0.2-0.3 mA.
The following performance characteristics for this probe were observed: 1) less than a ±10\% variation in the glycerol matrix ions (i.e., m/z 185) under normal operating flow; 2) upon repeated sample injections, less than a ±10\% variation in the reproducibility of peak areas with no significant deterioration in peak shape; 3) a 10-15 sec peak width at half height; 4) a significant reduction of memory effects between samples when compared to the commercial probe; and 5) improvement of the overall "sensitivity" by at least a factor of 10\(x\) greater than normally observed for static FAB.

An antiarrhythmic compound, ibutilide (U-70226, MW 384, C\(_{20}\)H\(_{36}\)N\(_2\)O\(_3\)S) was selected as a test compound. This drug represents a class of compounds of interest to our laboratory and the results obtained by CF-FAB could be readily compared to previous data obtained using static FAB. Approximately 500 ng of ibutilide yielded an MH\(^+\) 385 protonated molecular ion as the base peak under static FAB when scanning from 100 to 500 amu per sec. Using the CF-FAB probe, 50 ng of analyte yielded the same MH\(^+\) 385 protonated molecular ion along with structurally related ions at m/z 156, 214, 306, and 367 which were easily observed using the background subtraction features typically used for chromatographic separation.

In the MS/MS mode, CAD fragment ions were generated within the collision cell using argon as the target gas at a pressure of ca. 0.85-1.0 mtorr and applying a collision energy of -20 to -35 eV. A daughter spectrum of the MH\(^+\) 385 ion was obtained on 5 ng of ibutilide. Using SRM, m/z 385\(\rightarrow\)144, the lower limit of detection was approximately 50 pg.

Disadvantages associated with this technique are analogous to those associated with static FAB. For example, the well characterized effects of the matrix upon experimental results is still a consideration. In addition, CF-FAB has the constraints of very low flow rate which requires sample splitting for on-line HPLC operating at a flow rate of 0.5-2 ml/min. A better approach for isolating drug metabolites from biological samples, which would utilize the advantages of tandem mass spectrometry, may be off-line sample preparation prior to CF-FAB. Using a syringe pump, bypass valve, and manual injector, the LC system used in this study was configured to provide a convenient and rapid method for making loop injections of samples obtained from semi-preparative HPLC, solid phase extraction, or trapping techniques.

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1 Custom made in cooperation with Scientific Instrument Services, Inc.
**ANALYSIS OF PHARMACEUTICAL DEGRADATION PRODUCTS BY GRADIENT MICRO HPLC / DYNAMIC LIQUID SECONDARY ION MASS SPECTROMETRY (L–SIMS)**

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**Introduction:**
An important step in the development of pharmaceuticals is the characterization of degradants. When stressed by heat or light, pharmaceuticals partially degrade into new compounds which may influence product safety or efficacy. As part of the regulatory process, degradation products need to be identified and evaluated for toxicity. The characterization of trace amounts of pharmaceutical degradants can be complex due to the large excess of drug present in the sample. Furthermore, some degradants cannot be analyzed by traditional methods at the trace level due to instability or irreversible adsorption to glass. This study focuses on the application of gradient micro HPLC/dynamic L–SIMS for the rapid screening of crude degradants of the leukotriene antagonist compound LA–1 shown in Figure 1.

**Experimental:**
Degradation products were formed by extended exposure of compound LA–1 to concentrated HCl. In previous studies on related compounds this forced degradation method has facilitated the production of many naturally occurring degradants on a relatively short time scale (1–2 months).

Approximately 100 mgs of LA–1 was treated with 5 ml of concentrated HCl in a small petri dish, covered, and allowed to sit. Upon evaporation of the HCl solution to dryness, approximately 10 mgs of the resulting colored residue was removed and extracted with 3 mls of ethyl acetate. Compound LA–1 has low solubility in ethyl acetate and remained as a white precipitate. The partitioning of the colored degradants into the ethyl acetate could be visually observed. The ethyl acetate solution was decanted, taken to dryness under nitrogen, reconstituted in 100 ul of methanol, and concentrated to approximately 10–20 ul. Aliquots of this solution were then injected into the 60 nl fixed loop injector for micro HPLC/MS analysis. Conventional HPLC analysis of the colored solid residue fully dissolved in methanol showed that total degradants accounted for less than 1% of the total UV absorbance.

The hardware components of the micro HPLC/MS system are shown schematically in Figure 2. This system is somewhat unusual in that solvent gradients are formed at flow rates of a few microliters per minute without splitting. The solvent gradient is not linear; however, good day-to-day reproducibility was observed. UV detection was accomplished by using a conventional UV detector fitted with a specially manufactured capillary flow cell (LC Packings). This method was found to be more sensitive and easier to use than the on-column detection method.

Mass spectrometry was performed with a VG 70–VSEQ tandem hybrid mass spectrometer fitted with a screen/wick dynamic L–SIMS probe. A detailed drawing of the dynamic L–SIMS probe tip is shown in Figure 3. This probe design has provided stable long term (7 hour) performance with a variety of HPLC mobile phases. Good quality positive and negative full scan spectra were obtained for compound LA–1 at the 10 pmole level (6 ngs), and 1 pmole injections of LA–1 could be clearly observed in reconstructed ion profiles. The various micro HPLC and MS experimental parameters for the gradient experiments are summarized at the bottom of Figure 2.

**Results:**
The LA–1 degradant mixture was examined using gradient micro HPLC combined with both negative multiple ion monitoring (Figure 4) and full scan positive/negative ion switching mass spectrometry. In each of these experiments, the quantity of individual mixture components is conservatively estimated to be less than 10 ngs. The combined positive and negative ion data make tentative identifications possible (see Table 1). In some cases the fragmentation pattern is such that only generic identifications can be made. The major degradation pathways involve the formation of mono-, di- and tri-hydroxylated and hydroxy-keto degradants. The multiplicity of the various types of degradants is indicated by the number of peaks in the single ion profiles. The fine structure of the reconstructed ion profiles in Figure 4 is reproducible and can also be observed in the ion profiles derived from the full scan experiments. The portion of the chromatogram between 45 and 60 minutes is dominated by the hydroxy containing degradants. The sum of the various negative ion profiles for the hydroxy containing degradants is shown in Figure 4 and corresponds well with the UV absorbance trace.

**Conclusion:**
Micro HPLC/MS with positive/negative switching is a valuable technique for rapidly screening degradant mixtures for low level components. The direct (splitless) introduction of total microcolumn eluent into the mass spectrometer enables the full scan mass spectral analysis of degradants at the 1–10 pmole level. The screen/wick dynamic L–SIMS probe performs well with HPLC gradients, and very stable and reproducible results were obtained. The combination of positive and negative ion spectra facilitates the determination of molecular weight and structural features and is necessary to obtain maximum coverage of the chromatogram.
**Figure 1**
Compound LA-1
Molecular Weight = 561

Adducts Commonly Observed with water/acetonitrile/TFA/glycerol HPLC Mobile Phases
Positive ion mode $M+100 = (M+Glycerol-NH\textsubscript{2})^+$
Negative ion mode $M-113 = (M+TFA-H)^-$

**Figure 2**
Micro-HPLC Dynamic LSIMS System

**Table 1**
Degradants of LA-1
Analysis of $\pm$ micro HPLC/MS DATA

<table>
<thead>
<tr>
<th>Degradant</th>
<th>Polar Ion Molar Mass (amu)</th>
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<th>Negative Ions</th>
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<tr>
<td>trihydrated LA-1</td>
<td>307 (+)</td>
<td>306 (±)</td>
<td>305 (±)</td>
</tr>
<tr>
<td>trihydroxytaterj LA-1</td>
<td>305 (-)</td>
<td>304 (-)</td>
<td>303 (-)</td>
</tr>
<tr>
<td>hydrihydroxy LA-1</td>
<td>307 (+)</td>
<td>306 (±)</td>
<td>305 (±)</td>
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<tr>
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</tr>
<tr>
<td>608</td>
<td>630 (±)</td>
<td>607 (±)</td>
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**Figure 3**
Dynamic Liquid SIMS Screen/Wick Probe Tip

**Figure 4**
Illustration of Chromatographic Integrity.
Since its introduction, fast atom bombardment mass spectrometry (FABMS) has proven to be a powerful tool for the analysis of involatile and thermally labile compounds. The technique is simple to use and the generation of protonated ions of the type (M+H)+ allows for molecular mass determination for several classes of organic compounds. Many investigations concerning the basic processes involved in FAB have been conducted and several mechanisms have been forwarded to explain the features observed in the mass spectra. Of the mechanisms proposed, some invoke gas phase reactions while others are described in terms of solution phase processes. Solution phase processes in FAB ionization have been discussed in terms of preformed ions for ionic and zwitterionic compounds and fast atom beam-induced ionization for non-ionic species. Meanwhile, similarities in the ionic species generated in FAB and CI have been used as evidence of gas phase processes. In order to investigate the basic mechanisms involved in FAB ionization, systems in which anomalous (M-H)+ parent-molecular ions are produced have been extensively investigated in this laboratory. Cyclic acetals yield (M-H)+ ions and therefore offer an interesting system in which to compare the FAB results with that obtained in CI. In this work, a series of 2-phenyl-1,3-dioxolanes and several of their deuterated analogues have been systematically studied in FAB and CI and the mass spectra were compared in order to gain insight into the basic FAB ionization mechanism.

The (M+H)+ ion was observed to be the major peak in the molecular ion region of the CI mass spectra when NH₃, i-C₄H₁₀ and CH₄ were employed as reagent gases (Table 1 and Figure 1). It is interesting to note in the CI(CH₄) mass spectrum only, the presence of a significant ion at (M-H)+ which results from hydride abstraction from the neutral acetal species. CAD/MIKES experiments were performed to verify that the (M+H)+ ion was not the precursor of (M-H)+. Hence, in CI(CH₄) the (M+H)+ and (M-H)+ ion formation can be discussed in terms of competitive bimolecular gas phase reactions involving the neutral molecular acetals. In CI(NH₃, i-C₄H₁₀) experiments the (M+H)+ ion is predominantly formed by proton transfer and hydride abstraction is not a competitive process. This can be explained in terms of increasing hydride ion affinities (HIA) of the protonated reagent gas species: HIA for CH₅+, C₄H₉+, NH₄+ are 272, 231, 195 kcal/mol, respectively. From the CI(CH₄) mass spectra of the 2-d acetals it is observed that (M+H)+ is more intense than (M-D)+ which is assumed to be a consequence of the primary isotope effect where the fragmentation of the C-D bond is slower than that of the C-H bond.

From the FAB (magic bullet) mass spectra of the non-deuterated acetals (Table 1 and Figure 1) the (M+H)+ ion is observed to be dominant in the molecular ion region. For the 2-d acetals it is seen that the loss of D+ is strongly favored over H+ loss (Figure 1). This suggests that the (M+H)+ formation process in FAB is of a different energy to that in CI(CH₄). Interestingly, a (M+H)+ peak of significant intensity is also observed in the FAB mass spectra of the 2-d acetals which is again most likely a consequence of the primary isotope effect. Comparison of FAB results with CI requires a knowledge of the gas phase hydride ion affinity of the protonated magic bullet because in the gas phase model, the protonated matrix molecules are considered to act as the reactant gaseous species. The closest comparable HIA value to that of protonated magic bullet is for protonated methanol: HIA ((CH₃OH)H*) = 119 kcal/mol. Although the ((CH₃OH)H*) value may not be quantitatively accurate for HIA(protonated magic bullet), HIA(CH₅*) is over 150 kcal/mol.

In conclusion, the experimental evidence presented suggests that (M-H)+ ion formation in FAB is not the product of a gas phase process. One possible interpretation of the FAB results is that (M-H)+ and (M-H)+ ion formation occur in solution as competitive reactions. In Figure 2, the proposed gas phase and condensed phase potential energy diagrams for proton addition and hydride abstraction involving the cyclic acetals are displayed. In the gas phase, (M+H)+ formation is favored for CI(NH₃, i-C₄H₁₀) while competitive (M-H)+ formation was observed in CI(CH₄). For the solution phase process the activation energy barriers to the formation of (M+H)+ and (M-H)+ are known to be related to the solvation energies of the appropriate transition states for each reaction. If a three-charge-centre transition state is formed in the hydride abstraction reaction, this transition state would be better solvated than the proton transfer transition state, leading to a lower activation energy barrier for (M-H)+ formation with respect to (M+H)+ (Figure 2). Hence, this solution phase model explains the domination of (M-H)+ in the non-deuterated acetal FAB mass spectra which cannot be rationalized by bimolecular gas phase reactions, as indicated by the CI data.
The solution phase model presented in this study can also be used to explain the recent observation of the anomalous formation of both \((M-H)^+\) and \((M+Na)^+\) ions in the FAB mass spectra of some derivatized sugars containing ether groups\(^2\). In those systems, hydride abstraction by \(Na^+\) cannot compete with \(Na^+\) addition in solution while \((M-H)^+\) formation on reaction with the electrophilic matrix species in the condensed phase is predicted to be favored over simple protonation. Another interesting aspect of the application of the solution phase model to explain FAB ionization of cyclic acetals, is that the electrophilic matrix precursors to \((M-H)^+\) formation are not expected to be preformed in solution as both magic bullet and cyclic acetals are non-ionic. This suggests that the reactive electrophilic species are generated in solution from fast atom beam-induced ionization.

REFERENCES


TABLE I

<table>
<thead>
<tr>
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<th>CI HCN</th>
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</table>

\(\text{FIGURE 1}\)

PARENT-MOLECULAR ION REGION OF CI AND FAB MASS SPECTRA OF 4-HEXYL-2-PHENYL-1,3-DIOXOLANE AND ITS 2-d ANALOGUE

\(\text{FIGURE 2}\)

COMPARISON OF PROTON ADDITION AND HYDRIDE ABSTRACTION IN CYCLIC ACETALS IN GAS PHASE AND LIQUID PHASE

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Studies of reduction occurring during the FAB or LSIMS process have come to the conclusion that the phenomenon is due to interaction of the bombardment beam with the sample and/or the matrix. In our laboratory, this problem becomes quite significant when FAB MS is used for structure elucidation and identification of impurities in drug substances. GAZT was identified as a metabolite of AZT. In the course of the analysis, a peak was noted at 418 Da in the FAB mass spectrum which was shown to be the glucuronide of 1'-amino-2'-deoxy-thymidine (reduced GAZT) by MS/MS techniques. At first, this was thought to be another metabolite of AZT but subsequent NMR analysis and HPLC retention time data showed that reduced GAZT was not present in the metabolite samples and was an artifact of the FAB process. GAZT was subsequently synthesized in the Wellcome Research Laboratories and the purified material was shown not to contain any of the reduced impurity by NMR and HPLC analysis but did so by FAB mass spectrometry. At this point, we began a study into the conditions and causes of reduction of GAZT.

The LSIMS spectrum of synthetic GAZT in glycerol shows the (M+H)+ ion at 444 Da for GAZT and an ion at 418 Da for the reduced product. Mass spectra of GAZT in 1-thioglycerol and DTT/DTE (magic bullet) exhibit both ions but the reduced product appears at a lower level in both matrices compared to glycerol. The LSIMS mass spectrum of AZT and glutathione in these three matrices also exhibit this phenomenon. Reduction is also observed in the FAB MS of AZT where ions are observed in the matrices at 268 Da and 242 Da for AZT and reduced AZT respectively. Glutathione exhibits a protonated molecular ion at 613 Da and a reduced product at 308 Da which is the result of cleavage of the disulfide bond to produce two identical tripeptides. The amount of reduction product appearing in LSIMS mass spectra is greatest for glycerol and least for DTT/DTE for all three compounds. This is the opposite of what is expected since DTT and DTE are better reducing agents than glycerol. These data suggest that a phenomenon other than chemical reduction by the matrix is involved in this phenomenon. Yazdanparast, et al., also showed that the fast atom beam during FAB was essential for the production of reduction products for oxytocin in dithiothreitol/dithioerythritol (DTT/DTE). They also reported that different amounts of reduction occurred for peptides in thioglycerol and glycerol but did not pursue this observation further. Other researchers have concluded that the incident fast atom beam (FAB) or ion beam (LSIMS) is responsible for the reduction which occurs, but the nature of the matrix also plays a role in the amount of reduction observed.

To investigate this further, mixed matrices using glycerol and thioglycerol were used to obtain LSIMS spectra of GAZT and glutathione. Matrix mixtures of 75/25, 50/50, and 25/75 glycerol to thioglycerol were used. In all cases but one, the ratios are the same and are equal to the ratios obtained for pure thioglycerol matrix. This

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suggests that thioglycerol acts as an inhibitor to the production of reduced product. Recently, Kasakoff and Rye\textsuperscript{4} made the same observation for the FAB ionization of methylene blue in glycerol/thioglycerol mixed matrices. They conclude that the reduction observed in glycerol is due to free electrons produced by the bombardment beam and that thioglycerol is acting as an electron/radical scavenger which causes the reduction effect to be lowered in glycerol/thioglycerol mixtures. Kasakoff and Rye further suggest that for thioglycerol to exhibit the reduction products, another mechanism of reduction must be occurring. We have reached the same conclusion and support it as shown below.

Since reduction could also be due to chemical reduction involving the matrix, a hydrogenation catalyst, finely divided palladium on carbon, was added to the three matrices and mass spectra were obtained for GAZT and for glutathione. The data show that using the catalyst DTT/DTE produces a higher ratio of the reduced products for both compounds than does either thioglycerol or glycerol. Further, in the glycerol matrix, no GAZT or its reduction product could be observed while the signal for glutathione and its reduction product were quite weak, and no reduction in peak intensities was observed for the thioglycerol or DTT/DTE matrices. This is the order of matrices one would expect if the reduction were due to chemical interaction of a matrix and solute as discussed previously. Thus a second mechanism is present and it can be enhanced in the FAB/LSIMS process by introduction of a catalyst. However, this second mechanism is not involved in the glycerol matrix as shown by the lack of any enhancement of the reduction products by addition of a catalyst. To further prove that no reduction occurs in the glycerol matrix, glutathione was incubated at room temperature for five days with glycerol and glycerol plus Pd/C catalyst. HPLC analysis shows that the reduction observed in the glycerol matrix is not a result of the matrix alone resulting from a classical chemical reduction mechanism and that the reduction that is occurring is a result of the incident FAB/LSIMS beam. Using the information gathered from the experiments described above, we have developed experiments to determine the likelihood of reduction products occurring due to the FAB/LSIMS process rather than being components of the sample. Since the ratio of reduced to oxidized components is enhanced in glycerol compared to thioglycerol and DTT/DTE, analyzing the sample in more than one matrix and noting any differences in ratios should be a clue as to the origin of the reduction products. Further, addition of Pd/C to the DTT/DTE matrix should enhance those ions which are due reduction products of any other components in the sample. While this is not totally accurate in all cases, it does serve as aid in determining the source of impurity peaks observed in FAB/LSIMS mass spectra.

IDENTIFICATION AND CHARACTERIZATION OF AMINE CURED EPOXY PRODUCTS BY FAST ATOM BOMBARDMENT MS AND LIKED SCANS MS

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The curing of epoxy resins with amines to create high performance composites is of considerable commercial interest. In order to understand the properties and behavior of these materials, a fundamental knowledge of their chemistry is required. The overall understanding of the bulk properties is facilitated by the identification and characterization of the many products which are formed during the polymerization process. However, since most commercially used epoxy resins are polyfunctional and they are most often cured with polyfunctional amines, the final product is very difficult to characterize. As a means of simplifying the problem a model epoxy, methoxyglycidyl ether of Bisphenol A (MGEBA) has been cured with simple amines. The products of these simple reactions have been used in identifying key polymerization processes.

Initial mass spectrometric studies of the products formed between MGEBA and aromatic amines, have provided a wealth of information on the relationship between fragmentation patterns and molecular structure. Although the data base obtained from the aromatic amines curing process was helpful in identifying and characterizing the products of MGEBA cured with cyanamide, there were additional products initially formed, as well as some very different fragmentations which occurred with the cyanamide cured products. Because of the relatively small molecular size of the aliphatic amine, the products which would initially be formed would be much less sterically hindered and could allow for other interesting reactions not favored by the aromatics.

The initial reaction products of MGEBA and cyanamide were isolated by preparative liquid chromatography and analyzed by fast atom bombardment (FAB) mass spectrometry. The relationship between fragmentation and molecular structure of the initial products helped in the identification of the products subsequently formed. Because of the inherent greater reactivity of this system, any additional information that could be accumulated would be helpful in structure characterization. Therefore, in addition to the normal full scan mass spectrum a linked scan of each molecular ion, as well as other selected ions were acquired so that the proposed fragmentations could be confirmed.

The full scan mass spectrum of a representative product from the curing reaction mixture of MGEBA and cyanamide is shown in Figure 1a. It is interesting to note that this isolated compound is a product of dicyanamide, rather than cyanamide. The dimerization of cyanamide is much more rapid than the curing process, thus suppressing the cyanamide/MGEBA reaction. In addition to a strong molecular ion peak, shown in Figure 1a, the other most intense ion is m/z 439. The familiar fragmentations, involving the tertiary carbon of MGEBA or the amine functionality, observed with the products of the curing of MGEBA and an aromatic amine were not as evident in this system. Instead, the ion of m/z 439 is very highly favored. The collisionally activated decomposition (CAD) spectrum of m/z 681 is shown in Figure 1b and confirms the relationship of m/z 439 to the protonated molecular ion. The proposed ion structure for this ion is given in Figure 2. The large intensity of the m/z ion is attributed to the proposed resonance stabilization. Similar fragmentations involving cyclization of fragment ions were also observed for the other reaction products of this system.

The results of these studies as well as those obtained for aromatic amine curing begin to establish a database which relates molecular structure to mass spectrometric fragmentation patterns. The development of such a multidimensional data base combined with MS/MS techniques will aid in the further identification and characterization of increasingly challenging mixtures which are commercially more important.

Figure 1  a) Full scan FAB mass spectrum of representative product formed during MGEBA cyanamide reaction  b) CAD spectrum of m/z 681 (protonated molecular ion).

Figure 2  Proposed resonance stabilized structure of fragment ion m/z 439.
Polyyacetylenes and related synthetic polymers have been demonstrated to conduct electric current because of their extended series of conjugated double bonds (1). Potential applications of these polymers include use as organic semiconductors, components of electrolytic capacitors, or rechargeable batteries (2). In order to determine the factors that influence the conductivity of these polymers, accurate methods are needed to measure their chain length, molecular weight, and structure.

Field desorption (FD) mass spectrometry has been used to analyze oligomers and low molecular weight polymers such as poly(ethylenimine) (3). A newer technique than FD, fast atom bombardment (FAB) mass spectrometry has been applied to the analysis of a variety of compounds including biological polymers such as peptides and some synthetic oligomers and polymers (3). Compared to FD, FAB has the advantage of generating ions over a longer period of time, which facilitates collisional activation of selected precursor ions and then structural analysis of the product ions using tandem mass spectrometry. Recently, FAB mass spectrometry has been combined on-line with reversed phase HPLC in a system called continuous-flow FAB (CF-FAB) mass spectrometry (4). However, CF-FAB mass spectrometry has not previously been applied to the analysis of polyyacetylenes or other conductive polymers.

As part of our studies on the free-radical reactions of fluorinated alkynes and related compounds, we have been investigating the free-radical polymerization of 3,3,3-trifluoro-1-phenylpropyne to form a fluorinated polyyacetylene. We report here the synthesis and analysis of synthetic oligomers of 3,3,3-trifluoro-1-phenylpropyne (1) and its analog, 1-phenylpropyne (2) using gel permeation chromatography (GPC), FD mass spectrometry, CF-FAB LC/MS, and CF-FAB MS/MS.

The monomer, 3,3,3-trifluoro-1-phenylpropyne (1) was synthesized according to the method of Bumgardner and Bunch (5) and polymerized without solvent in a sealed vial at 90°C using benzoyl peroxide as a free radical initiator. The non-fluorinated analog, 1-phenylpropyne, was polymerized in a similar manner. Polymerizations were carried out with three different monomer/initiator ratios, 3:1, 10:1, and 20:1. Each crude reaction mixture was analyzed by GPC to determine the average molecular weight of the oligomers. Next, FD mass spectrometry and CF-FAB mass spectrometry and CF-FAB MS/MS were carried out on the crude reaction products to further characterize the oligomers.

Positive ion FD and CF-FAB mass spectra were obtained using a JEOL HX110HF double focusing mass spectrometer equipped with a DA5000 data system. The resolving power was set at 1000. Silicon emitters were used for FD, and a frit-FAB ion source at 45°C was used as the CF-FAB interface. B/E linked scans were carried out following collisional activation, and 5 to 8 scans were accumulated per analysis. The helium pressure in the collision cell in the first field-free region was adjusted so that the signal was attenuated 70%. LC/MS separations were carried out using an Applied Biosystems model 140A dual syringe pump, model 757 UV/VIS absorbance detector set at 450 nm, and Vydac C18 HPLC column (2.1 mm x 15 cm). The solvent system consisted of a 20 min gradient from 50:50:0.25 to 10:30:60:0.25 (v/v/v/w) water/methanol/ethyl acetate/3-nitrobenzylalcohol. The flow rate was 70 μl/min. The eluate from the HPLC column was split so that approximately 7 μl/min flowed to the mass spectrometer. Aliquots containing 20 μg of each reaction product mixture were injected onto the HPLC column per analysis. Because of the solvent split, approximately 2 μg of each mixture entered the CF-FAB interface.

The field desorption mass spectrum of oligomers of 3,3,3-trifluoro-1-phenylpropyne (monomer/initiator ratio of 10:1) is shown in Figure 1. Molecular ions of three series of oligomers were detected, one containing one hydrogen and one phenyl capping group (series A in Fig. 1), another containing two phenyl capping groups (series B), and a less abundant third series consisting of two hydrogen capping groups (series C). Both GPC and FD mass spectrometry provided similar average molecular weight information for the oligomeric mixtures and showed that a monomer/initiator ratio of 10:1 produced that highest average molecular weight. Ions within each series differed in mass by multiples of 170 mass units, with confirmed the molecular weight of the monomeric unit.
Figure 1. Positive ion FD mass spectrum of 2 µg of unpurified oligomers of 3,3,3-trifluoro-1-phenylpropyne synthesized using free radical polymerization with a monomer/initiator ratio of 10:1.

Because few sample ions of the unpurified reaction product mixture were detected using static FAB mass spectrometry, reversed phase HPLC was used to separate the oligomers in the mixture followed on-line by CF-FAB mass spectrometry. 3-Nitrobenzylalcohol in the mobile phase served as the FAB matrix. The reconstructed total ion chromatogram and selected ion chromatograms of the CF-FAB LC/MS analysis of oligomers of 3,3,3-trifluoro-1-phenylpropyne are shown in Figure 2. Like FD, CF-FAB mass spectrometry of these oligomers formed molecular ions without fragmentation. Therefore, CF-FAB MS/MS was used with collisional activation and B/E linked scanning to form structurally significant fragment ions. These analyses confirmed the presence of trifluoromethyl and phenyl substituents along the polyacetylene backbone. Ions corresponding to fragmentation of the polyacetylene backbone were also observed.

Figure 2. Reconstructed total ion chromatogram (TIC) and selected ion chromatograms for the CF-FAB LC/MS analysis of oligomers of 3,3,3-trifluoro-1-phenylpropyne (synthesized using a monomer/initiator ratio of 10:1).

We report here the first synthesis and characterization of oligomers of 3,3,3-trifluoro-1-phenylpropyne. In addition, the first CF-FAB LC/MS analyses of polyacetylenes are reported. CF-FAB LC/MS provided molecular weight and chain length information that was similar to FD analyses. However, CF-FAB LC/MS was also carried out, which provided additional structural information.

Mass Spectrometric Characterization of Conductive Polymers:

Mass spectrometry has become a viable technique for determining the molecular weight distribution and chemical structure of polymers with minimal sample preparation. Polyaniline\(^1\) and ring substituted\(^2\) polyanilines have been extensively investigated recently because of their moderately high electrical conductivity on doping with nonoxidizing Brønsted acids.

Attempts have been reported for the characterization of polyaniline and ring substituted polyanilines using field desorption (FD)\(^3\), laser desorption Fourier-Transform mass spectrometry (LD/FTMS)\(^4\) and secondary ion mass spectrometry (SIMS)\(^5\). Unfortunately, both LD/FTMS\(^4\) and FDMS\(^3\) provided little information on the chemical structure of these polymers. In SIMS, the highest mass detected in the negative ion spectrum contained only two monomer units; no direct information\(^5\) on the polymer chain structure was obtained from the positive ion spectrum.

In the present work, fast atom bombardment mass spectrometry is used to analyze poly(o-toluidine) (POT) of low molecular weight (m/z<5800) to evaluate its potential for the characterization of polymers of similar structure. Results show oligomer ions in the mass range of 400-2000 (Figure 1); only matrix ions are observed below m/z = 400. In the higher mass range (m/z 400-2000) ionic species detected are separated by the mass of a toluidine monomer (m/z = 105). An unexpected feature of the mass spectra is the observation of polymer series containing one oxygen atom per oligomer. This is further supported by MS/MS analysis of selected ions (Figure 2 and Scheme I). Several possible reasons for the presence of oxygen in the polymer will be presented.
CAD MASS SPECTRUM OF M/Z = 747

Figure 2

MS/MS ANALYSIS OF M/Z = 747

REFERENCES:
The use of filament winding techniques for the fabrication of laminate parts from carbon-fiber thermoplastic prepregs presents several problems for new high-performance resins which melt in the vicinity of 400°C. Small temperature excursions during processing may expose the resin to thermal degradation temperatures. In addition, oxygen present during processing may react with the hot resin. Consequently, it is important to know the effect of temperature and oxygen on the polymer in the vicinity of the process temperature.

10 to 20 mg samples of AS4/APC-2 carbon fiber/poly(aryl ether ether ketone) (PEEK) were isothermally heated for one hour at temperatures 35, 75, and 105°C above the recommended processing temperature (382°C) in nitrogen and 5 V% oxygen-in-nitrogen atmospheres. The latter was chosen to simulate the mildly oxidative environment likely during actual processing. Subsequently, vaporization/gas chromatography mass spectrometry (VapGC/MS) and evolved gas analysis mass spectrometry (EGAMS) were performed to determine the effect of sample treatment.

Sampling for Vap/GC analysis was performed by cryogenically trapping (Ref. 1) compounds desorbed from the prepreg during a 20 minute heat at 382°C. The chromatographic column was a Megabore DB-1 quartz capillary 30 m long by 0.54 mm ID. EGAMS was performed using 2 to 3 mg of sample crimped in gold solids probe sampling cups. The standard water-cooled solids probe of the ZAB mass spectrometer was heated from room temperature to 450°C at a rate of 10°C/min while mass spectra were continuously recorded.

Only minor differences were observed among the flame ionization chromatograms from samples treated at 417 and 457°C in the different atmospheres. No compounds were found in the mildly oxidized samples other than those present in the samples treated in pure nitrogen. There was a small decrease in the amount of material detected in chromatograms from samples treated in a mildly oxidative atmosphere compared to those treated in an inert atmosphere.

In contrast the flame ionization chromatograms from prepreg samples treated at 487°C for one hour were quite complex and difficult to compare. Additional compounds were formed in samples treated in the mildly oxidative atmosphere, and there was a greater quantity of products in the chromatogram from the mildly oxidized sample. Differences in the chromatograms were pinpointed by comparing the reconstructed ion currents of specific masses from the VapGC analyses. Masses 60 and 87 were very abundant in the reconstructed ion current plot from the sample treated in a mildly oxidative atmosphere. Differences in the EGAMS pyrolysis of the samples, mass spectra from the nitrogen treated sample were subtracted from mass spectra from the mildly oxidized sample at the equivalent probe temperature. A typical result is shown in Fig. 1. A positive mass peak indicates that the ion is more abundant in the mildly oxidized sample than in the sample treated in pure nitrogen, whereas a negative mass peak indicates the converse. Highlighted ions in the positive peak spectrum are not present in the sample treated in pure nitrogen.

Most of the ions seen in the pyrolysis of PEEK during EGAMS can be accounted for by simple cleavage of the polymer chain. Intra-chain cyclization about the ether portion of the chain to form furan compounds also may occur. The combinatorial tree below is coded as follows:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Moiety</th>
<th>Formula</th>
<th>Mass</th>
<th>Symbol</th>
<th>Moiety</th>
<th>Formula</th>
<th>Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>Ether</td>
<td>O</td>
<td>16</td>
<td>K</td>
<td>Carbonyl</td>
<td>CO</td>
<td>28</td>
</tr>
<tr>
<td>P</td>
<td>Phenyl</td>
<td>C₆H₄</td>
<td>76</td>
<td>D</td>
<td>Dibenzofuran</td>
<td>C₁₂H₈O</td>
<td>166</td>
</tr>
</tbody>
</table>

The sum of the masses of the coded parts plus 2 will equal the mass shown in the center of the table. The columns farther from the center are more hydrogen deficient. Almost all of the major ions observed as pyrolysis products are found in the combinatorial tree. The most abundant are hydrogen defi-
Fig. 1. Difference mass spectrum: pyrolysis products of mildly oxidized sample subtracted from nitrogen treated sample

A combinatorial tree of possible PEEK pyrolysis products

```
D
| OP 158
| POP 170
| KDP 196
| KPOP 198
| PKD 272
| PKPOP 274
| DOPK 238
| DOPKPOP 290
| DKD 362
| POPKD 364
| POPKPOP 366
| POPKPOP 368
| POPKPOP 369
| DOPK 454
| DOPK 456
| DOPKPOP 458
| POPKPOP 459
| POPKPOP 460
| POPKPOP 461
| POPKPOP 462
| POPKPOP 463
| POPKPOP 464
| POPKPOP 465
| POPKPOP 466
| POPKPOP 467
| POPKPOP 468
| KDPK 482
| KPOPKD 484
| KPOPKP 486
```

cient, indicating the presence of intrachain cyclized dibenzofuran in the major pyrolysis products.

Summary:
The presence of small amounts of oxygen has a marked effect upon degradation at 487°C, but less so at 457 and 417°C. The primary effect of oxygen at 487°C is to increase both the amount and the number of compounds produced during thermal treatment. EGAMS data indicate that at pyrolysis, samples treated in pure nitrogen and mildly oxidative atmospheres form many of the same products, except that they are more abundant in the oxygen treated sample. Several compounds such as D and DO are unique to the oxygen treated sample. This suggests that oxygen promotes the intra-chain cyclization process and the formation of lower molecular weight products.

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ANALYSIS OF PHOSPHITE POLYMER STABILIZERS
BY LASER DESORPTION FT/ICR MASS SPECTROMETRY

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Phosphite additives are used extensively at ~0.1% level as antioxidants in the polyolefin industry, and are conventionally detected by extraction, liquid chromatography, and/or Fourier-transform infrared spectroscopy [1]. However, FT/IR analysis of the intact polymer may be precluded for polymers which themselves have strong infrared absorption, and extraction-based analysis may not necessarily give an accurate measure of the additive concentration in the original polymer. Although mass spectroscopy has been applied with uneven success to the direct analysis of various nonvolatile additives [2,3], detection and quantitation of phosphite antioxidants has proved to be particularly challenging, because such species tend to fragment far more readily than other additives [3]. In this work, we report successful production and detection of abundant molecular ions from Ultranox® 626 antioxidant [bis(2,4-di-t-butylphenyl) pentaerythritol diphosphite] and its corresponding phosphate oxidation product, XR-2504, and in addition the phosphite additive, Weston™ 618 (distearyl pentaerythritol diphosphite) by Nd:YAG laser desorption Fourier transform ion cyclotron resonance mass spectrometry (LD/FT/ICR/MS). In particular, molecular ion peaks were seen for Ultranox® 626 antioxidant in polyethylene terephthalate, polypropylene, and acrylonitrile butadiene styrene (ABS) at additive concentrations as low as 0.1%, when the probe was heated to about 200 °C prior to laser desorption. Heating evidently increases the free volume of the polymer to facilitate laser desorption/ionization of the additives. LD/FT/ICR/MS thus offers a sensitive and accurate means for detecting nonvolatile phosphite additives at commercially typical concentration in solid polymers.

Experimental

All samples reported in this work were provided by GE Specialty Chemicals. Additive samples were (~20 mg in 1 ml of methylene chloride) were added dropwise to the probe tip. Subsequent evaporation of the solvent left a uniform thin sample film on the probe tip. Polymer samples were prepared by hot pressing to form a thin film which was then affixed to the probe tip. FT/ICR mass spectra were obtained at 3.0 tesla with an Extrel FTMS-2000 instrument. Laser desorption was performed with a Continuum Model YG-660A Nd:YAG laser operated at 1.064 μm, at a power level of ~50 mJ in 10 ns. Since laser irradiation typically produces ~1000 fold excess of neutrals over ions, best results were obtained by ionizing the laser-desorbed neutrals by means of an electron beam (70 eV) passing through the ICR ion trap.

Results

LD/EI/FT/ICR mass spectra of the Ultranox® 626 additive and the Weston™ 618 additive are shown in Figures 1 and 2. By optimizing the laser power and excitation parameters, we were able to generate abundant molecular ions. Figure 3 shows a mass spectrum of a 1:1 mixture of the phosphate additive, Ultranox® 626, and its corresponding phosphate—note the comparable ion yields of the two species. Figure 4 shows the spectrum of Ultranox® 626 (0.1%) in polypropylene. Although no fragments from the polymer are seen (due to its high molecular weight and the low laser power in this experiment), the additive is clearly detected at 0.1% level. [Work supported by N.S.F. (CHE-8721498) and The Ohio State University.]
Fig. 1. LD/EI/FT/ICR mass spectrum of Ultranox® 626 antioxidant (MW 604).

Fig. 2. LD/EI/FT/ICR mass spectrum of Weston® 618 (MW 732).

Fig. 3. LD/EI/FT/ICR mass spectrum of a mixture of Ultranox® 626 antioxidant and its oxidation product, XR-2504 (1:1 ratio).

Fig. 4. LD/EI/FT/ICR mass spectrum of Ultranox® 626 antioxidant in polypropylene (0.1%).

References

Stable fullerenes, C_{60} and C_{70}, were discovered in carbon cluster studies using photoionization and time-of-flight mass spectrometry (1,2). These fullerenes can be produced and extracted from graphite soots, as suggested by Kraetschmer, et al. (3). Fullerene molecules contain only carbons with non-planar sp2 hybridation and consist of twelve 5-membered rings with a differing number of 6-membered rings for each different fullerene. In the C_{60} molecule, the simplest form of fullerenes, every carbon of a 6-membered ring is shared with a 5-membered ring.

In this paper, we report the characterization of fullerenes by using various mass spectrometric techniques. The molecules are soluble in many organic solvents and sublimable above 300 °C. The onset temperatures for sublimation in MS vacuum were 300 and 360 °C, for C_{60} and C_{70}, respectively. With a desorption EI/CI probe, which can reach temperatures beyond 1000 °C, we also found higher fullerenes, most notably C_{90}.

Electron-impact ionization at 70-eV beam energy yields both singly-charged and doubly-charged molecular ions, indicating that the fullerene molecules are thermally and electronically stable. With high energy particle bombardment in plasma desorption, fullerenes also yield predominantly molecular ions (4). We also attempted to estimate first and second ionization potential by using a Finnigan TSQ-46B mass spectrometer. After calibrating with the known first IP of C_{60}, we estimate the first IP of C_{70} about 9 eV. The second IPs of C_{60} and C_{70} were estimated to be below 15 and 16 eV, respectively. In charge stripping experiments using oxygen as a collision gas, the second IP of C_{60} was measured to be 12.5 eV. When an inert gas, such as helium or argon, was used as a collision gas no charge stripping was observed (see Figure 1). Triply-charged ions were found in the collision-activation spectra of doubly-charged ions of fullerenes (Figure 2).

Chemical ionization (CI) using methane, isobutane and ammonia as reagent gases showed that the electron capture process is much more favorable than proton transfer and cation addition processes. In addition to increased sensitivity, electron capture yields much cleaner spectra than positive ion CI for fullerenes. The rather weak proton transfer spectra of fullerenes suggests that fullerenes are weak bases, at least in the vapor phase. Protonated molecules were observed in methane and isobutane CI, but not in ammonia CI. These results indicate that the proton affinities of fullerenes are between those of isobutane and ammonia.

Preliminary low resolution MS results indicate that unusual processes might also occur in negative ion MS. Negative CI using methane as a reagent gas showed (M + 14)-, (M + 16)- and (M + 30)- ions at m/z 734, 736 and 750, respectively. Using CD4 as a reagent gas, we see that these masses shifted to 736, 740 and 756, respectively (see Figure 3). These results suggest that (M + CH2)-, (M + CH4)- and (M + CH2 + CH4)- are present rather than (M + CH2)-, (M + O)- and (M + O + CH2)-. Confirmation of elemental composition of these ionic species by high resolution negative CI/MS is planned. When isobutane was used as a reagent gas, we observed (M + 58)- ion as well. Since C_{60} is a relatively large anion with high electron density, the reagent gas molecule might be able to attach to it, forming anion-molecule complexes.

Collision induced dissociation has been studied using a TSQ-46B tandem quadrupole mass spectrometer, which has collision energies limited to less than 30 eV. It was found that no dissociation was observed for cations and anions generated in the EI and positive/negative CI processes. However, with high collision energy in keV range using a tandem sector instrument, higher fullerenes, such as C_{70}, were found to dissociate into more stable lower fullerenes, such as C_{60} (Figure 4).
REFERENCES:

4. Plasma desorption mass spectra were obtained from W. Anderson of Lehigh University.

FIGURE 1
CHARGE STRIPPING OCCURS WHEN OXYGEN WAS USED AS A COLLISION GAS

FIGURE 2
CONSECUTIVE C2 LOSSES ARE OBSERVED IN THE COLLISION ACTIVATED SPECTRUM OF C60** WHICH ALSO SHOWS THE PRESENCE OF A TRIPLY-CHARGED MOLECULAR ION

FIGURE 3
SOME EVIDENCE OF ANION-MOLECULE COMPLEXES WAS OBTAINED BY USING LABELLED METHANE

FIGURE 4
HIGH ENERGY COLLISION (5 keV) UPON C70 YIELDS STABLE C60**
Analytical studies of transition metal oxide clusters are important due to the wide application of metal oxides, including use as catalysts and in microelectronic processes. Recently, mass spectrometric techniques have been applied in the study of gas phase clusters of molybdenum and tungsten oxides. Tandem mass spectrometry has been used in the structural characterization of heteropolyoxometalates. The objective of this study has been to examine various ionization techniques and instrumental configurations in studying the formation of metal oxide clusters. Particular attention has been paid to variations in cluster ion relative abundances using different techniques.

The highest number of clusters were observed in the chemical ionization spectrum for (MoO$_3$)$_n$, where $n=2$ to $12$, with a monotonic ion intensity decay after $n=3$. A closed ion source design required for the high pressure used in this ionization technique may have provided an optimum condition for the cluster formation. Another important factor is the fast heating rate which was applied to the direct exposure probe and provided a high oxide vapor concentration in the ion source.

The field desorption spectrum shows clusters from trimers to octamers for molybdenum oxide with a fast ion intensity decay after the hexamer. The absence of monomers and dimers with the presence of hexamers as the base peak in this spectrum suggest a special stability for trimers and hexamers. This ionization technique uses direct heating of the sample from an emitter in the presence of a high electric field which should provide the ionization potential necessary for a wide range of clusters. Several heating ramps were tested and the optimum rate was between 2 to 4 mA/min.

Field desorption mass spectrum of MoO$_3$ recorded on MS-1 of a JEOL HX110/HX110 tandem magnetic mass spectrometer which operated at an accelerating voltage of -10 kV, and a counter electrode voltage of +2.8 kV. The ion source temperature was 80°C. The spectrum is the sum of 6 scans in the profile mode with a 1.4 s scan time and a resolution of ±500. The oxides were loaded on benzotriflue activated carbon emitters from a methanol suspension by the dipping technique. The emitters were ramped to 30mA quickly followed by a ramp of 2 to 4 mA/min.
The most oxygen deficient cluster ions were observed using the SIMS ionization technique, where a 6 keV continuous neutral beam of xenon was used for sample desorption. The presence of these clusters suggests possible chemical reduction of the oxides. A very fast cluster ion decay after the trimer and the domination of reduced form of clusters for the tetramer are among other characteristic features of this ionization process. The probability for the presence of the reduced cluster increases with the cluster size. As the cluster mass increases, the ion signal for the oxygen deficient clusters becomes spread over an increasing mass range, effectively reducing the signal-to-noise ratio for any given peak in the cluster. Future comparison of these results to pulsed ionization beam experiments will provide a better understanding of ionization processes of this technique. The presence of oxygen adduct clusters is indicative of the oxyphilio nature of these transition metal oxides and several gas phase reactions with oxygen containing compounds have been demonstrated.

Laser desorption interfaced with time-of-flight instruments has been a popular and effective tool for cluster studies. An advantage of this technique is pulsed ionization which helps prolong the sample life-time and chemical state. The DLV mass spectrum of WO₃ at 355 nm shows cluster ions extending to hexamers. The base peak of the trimer and the presence of the oxygen rich ions are descriptive features of the spectrum. Polyoxometalates associate with cations in the solid state. Laser desorption studies of WO₃Na₄ were initiated in order to investigate formation of associated cation/anion clusters in the gas phase. The mass spectrum of this oxide shows similar clusters to WO₃, except for the abundant cluster series of [(WO₃)ₙNaO]⁺. This new cluster series dominates at higher cluster mass. The presence of WO₃Na⁺ ion suggests a chemical reduction which may be caused by the presence of the matrix. Future studies of transition metal oxides will investigate the interaction of different laser wavelengths and matrices.

Relative abundances of the cluster ions differ widely, depending on the technique used, and no "magic number" or special stability could be attributed to any of the cluster ions. The technique of chemical ionization using a quadrupole instrument appeared most effective in producing higher mass metal oxide clusters.

REFERENCES

ACKNOWLEDGEMENTS
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FOURIER TRANSFORM MASS SPECTROMETRY STUDIES OF TRANSITION METAL CLUSTER IONS PRODUCED BY FAST ATOM BOMBARDMENT

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Gas-phase transition metal cluster chemistry studies provide fundamental information which may be of use in surface, catalytic, and organometallic chemistries. Metal cluster ions are of particular interest, but are much more difficult to produce than atomic metal ions. The present work couples a universal cluster ion production method, fast atom bombardment (FAB), with a versatile method for studying gas-phase ion chemistry, Fourier transform ion cyclotron resonance mass spectrometry (FTMS). A variety of metal cluster ions can be studied by this technique.

The present study focuses on silver cluster ions. Silver clusters are important in photography, where photographic properties are dependent on the number of atoms in the cluster. Thus, the gas-phase chemistry of Agₙ⁺ may also exhibit size-related effects.

Silver cluster ions were produced by bombardment of silver foil, silver powder, silver oxide, and silver halides with a 10 kV xenon beam in an external FAB source. Electrostatic focusing was used to transfer the ions into the cell region of a Bruker CMS-47X FTMS. The reactivity of the mass-selected cluster ions was investigated with small molecules such as deuterium, oxygen, water, methanol, ethanol, methane, ethane, ethene, propene, benzene, and acetone.

One area of interest in the current study is the production of silver cluster ions. In the present experimental configuration, FAB on silver foil yields Agₓ⁺, x = 1-16. As is illustrated in Figure 1, Agₓ⁺ with odd x were seen in much higher abundances than ions with even x. These spectra are similar to those observed in FTMS studies of ions produced by laser desorption on silver oxide.(1) In contrast, xenon ion bombardment of silver foil in a magnetic sector instrument produces abundant even x clusters.(2) Thus, on the FTMS timescale the more stable odd x clusters (which have paired electrons) dominate.

The absolute ions intensities of Agₓ⁺, x = 1-5, may be greatly increased by applying HCl or H₂SO₄ to the Ag foil (Figure 2). The resulting spectra are similar to those obtained by FAB on silver oxide. In addition, silver halides produce Agₓ⁺ in high yields. The spectra also contain silver halide ions, as is illustrated in Figure 3 for AgCl. Silver halides proved to be the most convenient method for producing abundant small Agₓ⁺, therefore reactivity studies involved ions generated by FAB on silver bromide or silver chloride.

Ag⁺, Ag₃⁺, and Ag₆⁺ did not react with any of the small molecules involved in this study. This is not surprising since Ag⁺ is a stable 4s¹3d¹⁰ system, while the high abundances of Ag₃⁺ and Ag₆⁺ in the mass spectra indicate that these ions are also very stable. Ag₃⁺ is a closed shell system which is believed to have a triangular structure.(3) Previous studies have also found these ions to be unreactive with small alkanes, alkenes, and alcohols.(1)

In contrast to the odd x clusters, Ag₁⁺ was found to rapidly react with propene, acetone, and benzene to displace silver and produce AgL⁺ (L = reacting neutral). Slow reactions which could be quenched by argon were also observed with ethene and ethanol, while no reactions were observed with the other molecules involved in this study. The Ag⁺-Ag bond is weak, with a dissociation energy of 38 kcal/mol.(4) Thus, only species which have Ag⁺-L bonds that are stronger than 38 kcal/mole readily displace Ag from Agₓ⁺.
References

other fullerenes such as C\(_{2n}\), C\(_{g4}\), etc) can be produced\(^2\), numerous studies of the fullerenes are underway. While it was vaporization of graphite was due to a stable icosahedral structure, which became known as buckminsterfullerene or \"buckyball\". It was proposed that C\(_{60}\) might be responsible for spectral features in interstellar space known as the diffuse interstellar bands (DIB). With the recent introduction of a technique by which bulk quantities of C\(_{60}\) (and other fullerenes such as C\(_{2n}\), C\(_{g4}\), etc) can be produced\(^2\), numerous studies of the fullerenes are underway. While it has been established that C\(_{60}\) is not responsible for the DIB, it has been postulated recently that species such as C\(_{60}\)H\(^+\) may be important.\(^3\) Whether such species exist in the interstellar environment will depend on, among other factors, the proton affinity (PA) of the fullerenes. Recently, it has been reported that C\(_{60}\) can be protonated in the ion source of a mass spectrometer under conditions used for chemical ionization.\(^4\) This may have important implications for the existence of C\(_{60}\)H\(^+\) in the interstellar environment, as well as in the use of chemical ionization mass spectrometry for the analysis of fullerenes.

**EXPERIMENTAL**

The fullerenes used in this study were synthesized by a technique similar to that described by Krätschmer, et al.\(^2\) and separated by the technique described by Whetten and collaborators\(^2\). Chemical ionization mass spectra were recorded on a Finnigan TSQ-70 triple quadrupole mass spectrometer. The fullerenes were applied to a direct exposure probe as toluene solutions and desorbed by a current ramp. Various reagent gases were introduced into the source to form protonated and adduct ions. Proton affinities of the fullerenes were determined on an Extrel Fourier transform mass spectrometer. Reagent ions formed by electron ionization and ion-molecule reactions, and then isolated using SWIFT. Neutral fullerene ions were formed by volatilization from a heated probe. The proton affinities were determined by bracketing with various compounds of known proton affinity. Collision-induced dissociation mass spectra were obtained on the triple quadrupole instrument at voltages of 0-200 eV (lab energies). Xe was used as a collision gas under multiple collision conditions. Collision-induced dissociation mass spectra were also obtained on a ZAB-2PQ reverse geometry hybrid (BEGQ) mass spectrometer. Low energy collisions (0-500 eV) were carried out under multiple collision conditions in the quadrupoles with Xe as a collision gas. High energy CID spectra were obtained with MIKE scans, with 3-8 keV collisions using O\(_2\) as a target gas.

**RESULTS AND DISCUSSION**

Chemical ionization mass spectra of the fullerenes in the TSQ-70 showed that both C\(_{60}\)H\(^+\) and C\(_{70}\)H\(^+\) could be formed by protonation with reagent ions from isobutane (C\(_3\)H\(_7\)) and methane (CH\(_4\)). Adduct formation was far more extensive with isobutane reagent ions, suggesting that the proton affinities were similar. Despite the formation of \(\text{M+H}^+\) ions, considerable abundances of the radical cations of the fullerenes were observed, indicating that other types of reactions (charge transfer) occur in the CI source. Protonation was not observed with ammonia. Negative ion (electron attachment) were also obtained. C\(_{60}\) was found to have a higher electron capture cross section than C\(_{70}\), and negative ion CI is a more sensitive technique for the fullerenes. Negatively charged fullerene ions were observed out to C\(_{124}\). It was also found that reactions of C\(_{60}\) with oxygen could be observed with negative CI, with species such as C\(_{60}\)O\(^+\) (n=1-6) being detected. Similar reactions with fluorine (generated from SF\(_6\)) were also observed (C\(_{60}\)F\(_4\); n=1-2).

Proton transfer bracketing in the FTMS was used to determine the proton affinities of the fullerenes. The FTMS spectra of the proton transfer products of various reagent ions with the fullerenes showed an absence of charge transfer products, suggesting that the observation of the radical cation in the CI source is the result of reactions with species other than the major protonated reagents. The results of the proton transfer bracketing are shown in Table 1. The proton affinities of the fullerenes C\(_{60}\) and C\(_{70}\) are found to be between 204 and 207 kcal/mol. C\(_{70}\) is found to have a higher proton affinity than C\(_{60}\). The fact that proton transfer was observed between ammonia and the fullerenes in the FTMS and not in the high pressure CI source indicates that the fullerenes probably have a proton affinity nearly equal, but slightly higher than that of NH\(_3\) (multiple collisions or slow kinetic may affect the transfer process in the CI source).

Collision-induced dissociation mass spectra of the protonated fullerenes show an absence of significant fragments at energies of up to 200 eV. The primary fragmentation mechanism is the loss of H to form the radical cation. The CID spectra of fullerene adducts are dependent on the relative proton affinity of the adduct partner (A).
In general, fragmentation to lose AH*, A or AH (to form the radical fullerene ion) occurred. Significantly more fragmentation was observed with the radical cations of the fullerenes. Using Xe under multiple collision conditions (on the TSQ-70), C2 loss was observed in the 160-170 eV range (lab frame). At moderately higher energies (450 eV; Xe, multiple collisions) using the quadrupoles on the hybrid instrument, extensive fragmentation was observed (Figure 1). The enhanced abundances of certain carbon clusters (e.g. \(C_{11}, C_{15}, C_{19}, C_{30}\)) in the fragmentation spectrum corresponds well with the observation of similar "magic numbers" in studies of the formation of carbon clusters by laser vaporization. At high energy (4-8 keV; \(O_2\) target), similar results were obtained. In addition, charge stripping was observed with the singly charged fullerenes, and both stripping and reduction processes were observed in the multiply charged fullerenes.

Table 1. Results of Proton Transfer Bracketing Experiments

<table>
<thead>
<tr>
<th>MH* + C_n → C_nH* + M</th>
<th>PA (kcal/mole)</th>
<th>C_{60}</th>
<th>C_{70}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane</td>
<td>132</td>
<td>Y*</td>
<td>Y</td>
</tr>
<tr>
<td>Water</td>
<td>167</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Methanol</td>
<td>182</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Ammonia</td>
<td>204</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>C_{60}</td>
<td>-</td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>C_{70}</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexamethylbenzene</td>
<td>207</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Methylamine</td>
<td>214</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>N,N-dimethylamine</td>
<td>221</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Pyridine</td>
<td>221</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

*Y(N) indicates the proton transfer reaction did (did not) occur.

Figure 1. Low-energy collision-induced dissociation mass spectra of \(C_{60}^+\).

The physical and chemical properties of large carbon clusters, \( C_n \) (\( n > 40 \)), have been studied extensively over the past six years. The spherical nature of these clusters has been debated and inferred from both experimental and theoretical studies until the recent isolation and subsequent spectroscopic identification of the bulk fullerenes, \( C_{60} \) and \( C_{70} \). Although the \( n = 60 \) and 70 cluster ions are anomalously abundant ("magic numbers") in the mass spectrum, the other "magic numbers" have yet to be isolated. In a previous study\(^1\), the first ionization potentials (IPs) of \( C_n \) (\( n = 48 - 200 \)) were determined by charge-transfer bracketing of the carbon cluster cations, \( C_n^+ \), formed by the direct laser vaporization of graphite. The first IPs of the "magic number" clusters \( C_{50} \), \( C_{60} \) and \( C_{70} \) were determined to be approximately 0.5 eV higher than their neighboring clusters (e.g. \( C_{58} \), \( C_{62} \) etc.).

In this study, electron ionization (EI) of a mixture of \( C_{60} \) and \( C_{70} \) was used to generate the fullerene cations. In addition to the formation of singly-charged fullerenes, EI also generates a relatively high abundance of multiply-charged species (\( C_n^{x+} ; x = 2,3,4 \)). This allows the bracketing of the second and third IPs of the fullerenes by charge-transfer reactions in a Fourier transform ion cyclotron resonance mass spectrometer (FTMS). Following electron ionization, the \( C_n^{x+} \) of interest was mass-selected and then allowed to undergo radiative and collisional relaxation prior to the charge-transfer reaction period. The occurrence / non-occurrence of the charge-transfer reaction:

\[
C_n^{x+} + M \rightarrow C_n^{(x-1)+} + M^+
\]

was then determined as a function of the known IPs of neutral reactants, M. This technique allows the bracketing of the IPs of neutral or charged \( C_n \) species to within ± 0.1 eV to 0.2 eV depending upon the spacing of the neutral IPs (M) employed. An example of this procedure is illustrated in Figure 1 which shows the mass spectrum obtained following the isolation, thermalization and reaction of \( C_{60}^{2+} \) with fluorobenzene (IP = 9.20 eV). The observance of the charge-transfer reaction indicates that the IP(\( C_{60}^+ \)) or 2nd IP(\( C_{60}^+ \)) = 9.20 eV. The first, second and third IPs of \( C_{56} \), \( C_{60} \) and \( C_{70} \) were bracketed by this procedure to yield the following results:

<table>
<thead>
<tr>
<th>( C_n )</th>
<th>1st IP (eV)</th>
<th>2nd IP (eV)</th>
<th>3rd IP (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{56} )</td>
<td>7.1 ± 0.1</td>
<td>9.1 ± 0.1</td>
<td>11.2 ± 0.2</td>
</tr>
<tr>
<td>( C_{60} )</td>
<td>7.6 ± 0.1</td>
<td>9.7 ± 0.2</td>
<td>11.2 ± 0.2</td>
</tr>
<tr>
<td>( C_{70} )</td>
<td>7.6 ± 0.1</td>
<td>9.2 ± 0.1</td>
<td>11.2 ± 0.2</td>
</tr>
</tbody>
</table>

The first IPs determined here are in agreement with the previous results using direct laser vaporization (DLV) of graphite as the \( C_n^+ \) source. This illustrates the similarity of the energetics/structures of \( C_n^+ \) formed by DLV of graphite and EI of gas-phase fullerenes. It was observed that \( C_{60}^+ \) reacted faster with m-toluidine (IP = 7.50 eV) than did \( C_{70}^+ \). This suggests that IP(\( C_{60}^+ \)) > IP(\( C_{70}^+ \)). To verify this, \( C_{60} \) and \( C_{70} \) were used as the neutral charge-transfer reagent in the reaction of \( C_{60}^+ \) and \( C_{70}^+ \) as shown in Figure 2. Charge transfer occurs in both
reactions but is clearly much faster from $C_{60}^+$ indicating that the IPs are nearly equivalent but $IP(C_{60}) > IP(C_{70})$.

The second IPs are only 1.5 - 2 eV higher than the first IPs which is consistent with their large abundance in the El spectrum, but are much lower in comparison to estimated second IPs of polycyclic aromatic hydrocarbons (e.g. 2nd IP(C$_{24}$H$_{12}$) = 13.3 eV). The trend in which the magic number clusters (C$_{60}$ and C$_{70}$) exhibit greater stability (higher IP) is also observed in the second IPs; 2nd IP(C$_{60}$) > 2nd IP(C$_{70}$) > 2nd IP(C$_{56}$). The third IPs are only ~2 eV higher than the corresponding second IPs which illustrates the high stability and electron delocalization / aromaticity of the fullerenes. The third IPs of C$_{56}$, C$_{60}$ and C$_{70}$ are indistinguishable, however, by the charge-transfer bracketing technique (11.2 ± 0.2 eV). In addition to charge transfer, several other interesting reactions occur for the triply-charged fullerenes including H$^+$ transfer,

$$C_n^{3+} + C_3H_8 \longrightarrow C_nH^2^+ + C_3H_7^+$

and O$^-$ transfer,

$$C_n^{3+} + CH_3NO_2 \longrightarrow C_nO^{2+} + CH_3NO^+.$$  

Figure 1. Reaction of $C_{60}^{2+}$ with $C_6H_5F$

Figure 2. Reaction of $C_{60}^+$ (top) and $C_{70}^+$ (bottom) with neutral fullerenes (C$_{60}$ and C$_{70}$)

FLOWING AFTERGLOW STUDIES OF BUCKMINSTERFULLERENE ANIONS

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Buckminsterfullerene anions \( \text{C}_{60}^- \) and \( \text{C}_{70}^- \) have been formed by electron attachment in a flowing afterglow-triple quadrupole mass spectrometer. \( \approx 10^8 \) collisions with He in the flowing afterglow thermalize the ions, allowing room temperature reactions to be studied.

Both fullerides are unreactive with a variety of acids including \( \text{CF}_3\text{COOH} \), an extremely strong gas-phase acid (\( \Delta G_{\text{acid}} = 317 \text{ kcal/mol} \)). This indicates either that \( \text{C}_{60}^- \) and \( \text{C}_{70}^- \) are weak Bronsted bases, or that there is a substantial kinetic barrier to proton abstraction. Both explanations are consistent with the expected delocalization of the excess electron in these ions. The fullerides react slowly with \( \text{BF}_3 \), a very strong Lewis acid. This demonstrates that the fullerides are weak Lewis bases as well.

\( \text{C}_{60}^- \) and \( \text{C}_{70}^- \) react with \( \text{NO}_2 \) to form \( \text{C}_{60}\text{NO}_2^- \) and \( \text{C}_{70}\text{NO}_2^- \) with effective bimolecular rate constants of \( 1.2 \times 10^{-10} \) and \( 2.1 \times 10^{-10} \text{ cm}^3/\text{s} \), respectively. The rates correspond to reaction efficiencies of 19% and 33%. No further addition of \( \text{NO}_2 \) is seen, consistent with radical coupling of \( \text{NO}_2 \) and the fulleride anions. The rate constant is essentially independent of the argon buffer gas pressure over the range 0.1-0.5 Torr, suggesting that collisional stabilization of the adduct is saturated at these pressures.

\( \text{C}_{60}^- \) and \( \text{C}_{70}^- \) do not react with \( \text{NO} \) within the sensitivity of the experiment. Since \( \text{NO} \) is a doublet like \( \text{NO}_2 \), it is clear that radical character alone is insufficient to allow reaction. It is likely that high electron affinity leads to greater reactivity: \( \text{EA(NO)} = 0.03 \text{ eV} \), \( \text{EA(NO}_2) = 2.27 \text{ eV} \).

\( \text{NO}_2 \) does not extract an electron from \( \text{C}_{60}^- \) or \( \text{C}_{70}^- \), indicating that the vertical ionization energy of these molecules is greater than 2.27 eV. This is consistent with the work of Smalley et al. (Chem. Phys. Let. 1987, 139, 233), who estimate the electron affinity of \( \text{C}_{60}^- \) as 2.6-2.8 eV.

The reaction of \( \text{C}_{60}^- \) and \( \text{C}_{70}^- \) with \( \text{O}_2 \) shows a wide variety of products, but the majority of these are likely due to impurities. The reaction of \( \text{C}_{60}^- \) and \( \text{C}_{70}^- \) is at most very inefficient.
Figure 1. Mass spectra showing reactions of $C_{60}^-$ and $C_{70}^-$ with NO$_2$ at NO$_2$ flow rates (10$^{-3}$ STP cc/s) of (a) 0.8, (b) 9.4, and (c) 43.

Figure 2. Attenuation of $C_{60}^-$ and $C_{70}^-$ as a function of NO$_2$ flow rate. The reaction rates can be calculated from the slope of the decay curve.
IDEA:
A design is presented for a mass spectrometer based on the deflection of a pulsed ion beam with a time dependent electric field. By choosing the proper time dependence for the electric field, exact energy focussing can be obtained. This design is an alternative to a time of flight spectrometer. The design based on pulsed deflection would not require a bandwidth of the order of 100 MHz for the electronics, as required for a time of flight spectrometer. This could simplify the electronics. The spectrometer could deal with ions having an extremely broad distribution of initial energies.

The transverse displacement is

\[ y = \int_{t_d}^{t_l} \int_{t_d}^{d} \frac{e V(t')}{md} dt' dt \]

where

\[ td = \frac{L}{\sqrt{2eVa/m} + vo} \]

Choose a deflection voltage of the form

\[ V(t) = \frac{b}{t^2} \]

where \( b \) is a constant. Substituting back into the integral, one can simplify to

\[ y = \frac{eb}{md} \left( \frac{l}{L} + \ln \left[ \frac{L}{l+L} \right] \right) \]

Remarkably, the solution is completely independent of initial velocity. One can also solve for the exit angle

\[ \tan(\theta) = \frac{eb}{mdL} \left( \frac{l}{L} \right) \]

The required deflection voltages are of the same order as the acceleration voltage. The divergence of the deflection voltage as \( t \to 0 \) simply means that there is a lower mass limit to the deflected ions for a given voltage source.

SIMION calculations confirming the independence of deflection from initial energy are presented.

A deflection voltage of the form of a decaying exponential can be used to approximate a \( 1/t^2 \) voltage for a limited mass range. Such a voltage could easily be obtained from a discharging capacitor.
A heavy cluster primary ion beam source is being studied for desorbing large biomolecules from liquid matrices. The objectives of this research are threefold: increase desorbed secondary molecular ion yields, decrease chemical noise in FAB spectra and gain insight for contributing to existing theories that model desorption of macromolecules. The advantages of using heavy cluster primary beams in place of monatomic ion/atom or small molecular ion beams are several. Because clusters are multiply charged, impact energies up to 1 MeV or more are possible using only moderate acceleration voltages (up to 20 kV). At the same time, the impact energy per cluster particle is sufficiently low (1-3 eV) to prevent excessive radiation damage and subsequent ionization of damaged matrix material. Multiply charged heavy clusters are formed by electrohydrodynamic (EH) atomization of conductive aqueous methanol and glycerol mixtures in vacuum.

For this study, cluster beams were focused onto a JEOL HX100HF magnetic sector FAB stage. Negative ion spectra for a peptide of m/z = 2394 generated by cluster bombardment and a standard +6kV JEOL xenon gun are compared in Fig. 1 and Fig. 2. Clusters used in this study were formed at +15.3 kV potential from a 30% glycerol:70%water solution. Immediately apparent in the spectra comparison is the dramatic reduction in chemical noise as seen in the cluster spectra (Fig. 2), especially in the region of the doubly charged ion at m/z = 1197. Although the total ion yields are similar in this case, the signal-to-noise is vastly improved. Studies using other peptides show a similar reduction in the chemical noise background and, additionally, little or no fragmentation products, demonstrating the excellent potential of heavy cluster desorption for a "soft" particle-induced desorption technique exhibiting high signal-to-noise. In fact, using a FMRF-Amide-Like neuropeptide (m/z = 1243), the molecular ion was the major peak in the spectrum since the yield of low mass secondary matrix ions was greatly reduced compared to their predominant production using primary ion beams.

We conclude from these studies that, when using primary ion beams, chemical noise in FAB spectra is due to radiation damage in localized matrix regions with subsequent ejection of secondary matrix ions representative of the damage products. On the other hand, chemical noise using a primary heavy cluster beam is greatly reduced since the large cluster energy (MeV's) is distributed over a wide matrix impact area. Although sufficient to eject preformed ions, the low average impact energy per cluster particle (3 eV) minimizes both matrix damage and the desorption of damaged matrix ions. Presently, cluster beams generated by the EH method are accompanied by a small ion component in the beam. By reducing or eliminating this ion component in the cluster primary beam, achievement of even lower chemical noise and improved signal-to-noise in FAB spectra is predicted.
Fig. 1. Negative Ion Spectrum Using Primary Ion Beam Source

Fig. 2. Negative Ion Spectrum Using Heavy Cluster Beam Source
ON THE STRUCTURE AND FORMATION MECHANISM OF IONS AT m/z 130 OF CHALCONE

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The use of mass spectrometry for structural elucidation of flavonoids has received considerable attention (1). The major fragmentation pathways and general rearrangement processes of these compounds can determine, in some cases, the type of flavonoids and establish the position of substituents on the molecular skeleton.

In particular, mass spectrometry of chalcone received considerable attention. The general fragmentation pattern and some intimate EI-induced decomposition mechanisms have been already discussed by us by ITMS measurements (2).

Still, in literature are present some discrepancies among some decomposition routes, as for example those leading to ions at m/z 130. Thus while Van de Sande et al. (3) sustained that such ion originates from a cyclic moiety and it is structured as a, Rouvier et al. (4) in an earlier investigation, based on substituted chalcones, proposed structure b (see figure 1). It is worth noting that structures a and b involve the presence of the two different phenyl groups of the original molecule. In the present study, in order to gain definitive information on both formation mechanism and structure of ions at m/z 130, we have investigated differently labelled (D, $^{13}$C, CH$_3$ and F) compounds 1-11 (see...
figure 2) by means of metastable ion studies (B$^2$/E linked scans and MIKES) by a double focus VG ZAB 2F instrument and collisional experiments performed on an ion trap device (Finnigan ITMS).

$$\text{Figure 1}$$

References


The non-enzymatic reaction between glucose and free amino groups of proteins is the first of a complex series of reactions (Maillard reaction) determining the formation of brown-yellow, fluorescent, insoluble products: the Advanced Glycation End Products (AGE). Their accumulation on long-lived proteins is considered one of the most important damage factors leading to diabetic complications (1).

Many efforts have been devoted by different groups to the identification of some of these products. These studies must necessarily pass through a degradation in well controlled conditions of the proteins, leading to the formation of low molecular weight species coming from the substructures originating from the interaction freeaminogroups-glucose and for these aim acid and enzymatic hydrolysis are currently employed. In this contest 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole (FFI)

was proposed as an AGE as it was identified among in vitro glycated and furtherly acid hydrolyzed albumin and polylysine (2,3).

Collisional spectroscopy was proved by us to be effective for an easy identification of FFI in different substrates (4) without any sample pretreatment or chromatographic separation with a detection limit in the ppb level. By such approach we proved the absence of FFI in in vivo glycated proteins (collagen of diabetic rats), demonstrating the unreliability of the alternative analytical procedures employed for FFI quantification (5). In particular RIA methods led to misleading results due to the poor specificity of the developed antibody reactive not against whole FFI, but against a substructure of FFI, i.e. the furoyl moiety.

Parent ion spectroscopy carried out on furoyl cation (m/z 95) accounted for the RIA results: in fact eight different furoyl containing compounds were easily evidenced and further identified by daughter ion spectroscopy performed on the related molecular ions (6). Such approach demonstrated the high specificity of collisional spectroscopy: the results that by RIA were ascribed to the presence of a single compound, by collisional experiments were proved to originate from eight structurally identified compounds, different from that invoked as real AGE marker.
In order to clarify the FFI origin (7) we performed enzymatic hydrolysis on in vitro and in vivo glycated proteins. In both cases FFI was not found, proving that it must be considered an artifact coming from acid hydrolysis (8, 9).

In the frame of a mechanistic study on AGE production, based on reaction between protected lysine and glucose, collision spectroscopy was successfully employed in the structural characterization of some reaction products, directly carried out in the reaction mixture (10).

References
1) Monnier V.M. and Cerami A. Clin Endocrinol Metab 11, 431 (1982).
EVIDENCES FOR GAS-PHASE, ION-MOLECULE REACTION AS RESPONSIBLE FOR CROWN ESTER BEHAVIOUR IN FAB CONDITIONS

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Fast atom bombardment (1) and liquid secondary ion mass spectrometry (2) have become ionization techniques currently employed, due to their use in mass spectrometric analysis of large and/or highly polar molecules (3). When the samples under study are neutral moieties, they usually undergo extensive protonation and/or cationization reactions which some Authors proposed to happen in the condensed phase while some others sustained to occur in the "selvedge" region, an high density gas just close to the matrix surface (4,5). However, the sequence of desorption/ionization phenomena can be dependent from the compounds under study (6).

In previous paper we described the El mass spectrometric behaviour of some crown esters (7) that show the formation of [M+H]+ and [M-H]+ ions. Such unusual behaviour was rationalized in terms of intrinsic basicity and intrinsic acidity of their M and M+- species respectively. For such reason we thought of interest to undertaken a study on FAB mass spectrometry of compounds 1-3 in different operative conditions in order to gain information on the FAB ionization mechanism.

Compounds 1-3 lead also in FAB conditions to [M+H]+ and [M-H]+ species in ratios very close to those observed in El conditions, together with abundant fragment ions. This behaviour can be justified by three different mechanisms:

i) Parallel protonation and hydride abstraction reactions occurring in condensed phase and further desorption of the preformed ions;

ii) FAB activation leading to unstable M+ species in the matrix, which reacts as in i) but in the condensed phase, thus giving rise to preformed ions [M+H]+ and [M-H]+;

iii) FAB activation firstly leading to the desorption of intact molecule and/or M+ ions, which in the "selvedge" region undergo gas-phase, ion-molecule reactions responsible for the [M+H]+ and [M-H]+ ions production.

In order to obtain data on the possible mechanism(s), a series of experiments was undertaken.

1: R = CH₂-CH
   CH₂-CH

2: R = (CH₂)₇

3: R = (CH₂)₈
The presence of matrix as well as its acidification do not lead to any variation in the $[M+H]^+/[M-H]^+$ ratio. These results lead to exclude hypothesis i) and ii), otherwise the formation of preformed ions in condensed phase should be easy detected. Metastable ion studies demonstrate that $[M-H]^+$ ions are not unimolecular decomposition products of $[M+H]^+$ ions.

Adding sodium or potassium chlorides in the glycerol solutions of 1-3 an easy formation of $[M+Na]^+$ and $[M+K]^+$ was observed together with general decrease of $[M+H]^+$ ions. Such data indicate that mechanism iii) seems to be the more reasonable one. The results here obtained support that in this case the FAB data must be rationalized in term of "gas-phase" explosion model.

References
MASS SPECTROMETRIC INVESTIGATION ON MONO-, DI- AND TRI-CARBOXYLIC DERIVATIVES OF PYRROLE

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Pyrrole carboxylic acids have been found among the products of chemical degradation of melanins. The formation of these acids may be explained by the presence of carboxylated pyrrole units in the polymer structure or by assuming that carboxylated pyrrole units are linked through a carbonyl group to position 4 or 7 of indolequinone units (1). Since identification of these acids among the degradation products of melanins may give important structure indications, this study is aimed to their structure characterization by mass spectrometry (2,3). Until now, the mass spectrometric behaviour of pyrrole 2-carboxylic acid (compound 1) only has been described (4) and we will discuss it together with those of compounds 2-6.

The two pyrrole monocarboxylic acids (compounds 1 and 2) behave quite similarly leading to the same fragmentation pattern, also if clear differences are present in relative abundances of molecular and fragment ions. Deuterium labelling experiments proved that the primary H$_2$O loss implicate, for both 1 and 2 together with the carboxylic OH group, aminic hydrogen and H atom linked to carbon. The ratios M-D$_2$O/M-DHO are different for 1 and 2. Both primary COOH and CO$_2$ loss are also observed.

The pyrrole di-carboxylic acids (compounds 3-5) show an analogous decomposition pattern. Again the primary CO$_2$ loss is responsible for the base peak of all the isomers. It must necessarily lead for 5 to the M$^+$ of pyrrole 2-carboxylic acid, while for 4 to the formation of M$^+$ of pyrrole 3-carboxylic acid.

In the case of compound 3, two possibilities can be present: i.e the CO$_2$ loss could lead to the formation of pyrrole 2- and/or 3-carboxylic acids. The collisional spectrum of M-CO$_2$ ions of compound 3 results wholly superimposable to that of M$^+$ of compound 1 so proving that the CO$_2$ loss originates from the carboxylic moieties in position 3.
The primary H₂O loss is present for all the compounds, but for 3 and 4 the formation of anhydride species can be invoked, for compound 5 a fragment ion of the same structure already proposed for [M-H₂O]^+ of 1 and 2 must be considered. A clear difference exists among the CAD-MIKE spectra of compounds 3-5 in the relative abundance of [(M-H₂O)-OH]^+ ions. These species results more abundant for 3 and practically absent for 4. The [(M-H₂O)-OH]^+ ion is present also for 5, but its formation mechanism must be completely different from that proposed for 3.

Finally, for compound 6 the primary metastable supported fragmentation pathway is due to CO₂ loss, leading to ion at m/z 155, isomeric to M⁺ of compounds 3-5. Again, different structures of [M-CO₂]^+ ion can be proposed but simply looking at its further fragmentation processes, some information can be easily gained. In fact ion at m/z 155 further decomposes through CO₂ and H₂O losses, as observed for M⁺ of 3-5. But, in the present case, a new fragmentation route, consisting in the CH₂N⁻ loss, is present, suggesting that the structure of [M-CO₂]^+ ion for 6 is different from those of M⁺ of compounds 3, 4 and 5, i.e it must be that of M⁺ of pyrrole 2,4 dicarboxylic acid.

References

The Role of Fluorine in the Fragmentation Pathways of Some Fluorosubstituted Barbituric Acids

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Pursuing our interest in the mass spectrometry of fluorine-containing compounds, we have compared the behaviors of barbituric acid (compound 1), 1,3-dimethyl barbituric acid (compound 2) and of some fluorinated derivatives (compounds 3-8). This investigation was carried out by electron impact (EI) ionization, as well as by both positive ion and negative ion fast atom bombardment (FAB) in order to gain information on the relative stability of protonated and deprotonated molecular species, together with extensive metastable ions study. All mass spectrometric measurements were performed on a VG ZAB 2F operating both in EI (70 eV, 200 uA, ion source temperature 130°C) and in FAB (3 keV Xenon atoms bombarding glycerol solutions of the samples) conditions. Metastable ions study was achieved by means of mass analyzed ion kinetic energy spectroscopy (MIKES).

Compounds 1 and 2 lead to a common fragmentation pattern. The values as obtained by MIKE spectra of M⁺ of 1 and 2 led us to propose molecular species in an open form originating from the cleavage of a ketoimidic bond with the formation of an \( \delta \) distonic ion:

\[
\begin{align*}
&\text{O} = \text{C} + \text{CR}^* + \text{F} + \text{C} \equiv \text{N} + \text{C} \equiv \text{N} + \text{H} \\
&\text{R}^* \quad \text{R}^* \quad \text{R}^* \quad \text{R}^*
\end{align*}
\]

From this distonic ion, arise all the fragment ions through the indicated cleavages in a position to the carbonyl groups and most of the fragment ions as well can be written in an open form corresponding to a \( \delta, \delta' \) or \( \delta '' \) distonic ion. In some cases, both complementary ions are detectable. However, a different behavior for 1 and 2 is well evidenced by cleavage 1 due to the presence of H rearrangements only for compound 2; this can be explained by the presence of the two N-methyl groups.
For compounds 3 and 4, molecular species can be proposed structured as the following distonic ion:

\[
\text{O}_2\text{C-C}_2\text{N}-\text{C}^+\text{N}^+\text{C}^+\text{CF}_2
\]

originating not from the already described cleavage of the chetoimidic bond, but from the rupture of F\text{2C-C} bond. The driving force of this alternative cleavage must be the electron withdrawing power of fluorine, as well as the particularly high stability of the -\text{CF}_2- radical. Also in this case, cleavages in \(R^2\) to the keto groups of the molecular species are responsible for the most of fragment ions; however, while for 1 and 2 complementary ions were often present, cleavages of the distonic ions in the case of 3 and 4 lead mainly to fluorine containing moieties. For compound 4, the occurrence of H rearrangement in fragmentation routes 2 and 4 can be related to the presence of N-methyl group. In the case of 5-fluoro, 5-alkyl derivatives 5-7, a common fragmentation pattern can be proposed. Most of cleavages, originating from molecular ion in distonic form, lead again to different distonic fragment ions:

\[
\text{O}_2\text{C-N}^+\text{C}^+\text{N}^+\text{C}^+\text{CF}^+\text{R}^2
\]

However, not all the fragmentation products of 6 and 7 can be rationalized via the common distonic ion proposed for the molecular species of 5-7. Hence we propose for 6 only, also an alternative rupture of HN-CO bond analogously to unfluorinated compounds 1 and 2. For compound 7 as well some fragment ions must reasonably come from an alternative rupture due to non equivalence of C\text{5-C}4 and C\text{5-C6} bonds. Hence another distonic ion can be invoked originating from ring opening between C\text{5} and C\text{6}. Finally, for compound 8, the 5-fluoro,5-phenyl derivative, the most of its fragmentation pattern can be justified by cleavage of C\text{5-C}0 bond, but the further decomposition processes are mainly due to the formation of highly stable phenyl containing ionic species.

As far as FAB measurements, compounds 1 and 2 under positive ion FAB conditions lead to protonated molecular species which are the base peak of the spectrum. Only three decomposition pathways involving ring cleavages are observed. Furthermore, a primary water loss, never detected in El, is present in both normal and MIKE spectra of MH+ species. The observed losses from MH+, lead us to propose as favoured protonation site the carbonyl oxygen. For FAB data, the intermediacy of a distonic cation can not easily be proposed.

Compounds 3 and 4 also show abundant MH+ ions and a particularly favoured water loss. The observed cleavages still indicate that also in FAB conditions the F\text{2C-C} bond is weaker than the H\text{2C-C} one. Compounds 5-7 still show the primary water loss and a particularly favoured primary F radical loss. The base peaks for both are due to FR\text{1-R}R\text{2C} loss. Compound 8 behaves quite similarly to 5-7, showing water, F, R\text{1-R}R\text{2C} losses.

Negative ion FAB spectra show for all compounds a base peak due to [M-H] \(-\) species, giving account for a particularly high stability of such ion. Further observed fragmentation is very poor: loss of CO is present for 1-8 while [(M-H) \(-\) (CONCO)]\(-\) particularly abundant for 5 and absent for 4 seems to indicate that for 1-3 and 5-8 proton abstraction originates from NH moieties. The lack of ring cleavages other than CO loss is to emphasize.
Electrohydrodynamic disintegration of liquids and ion formation in electrospray MS

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The ion formation in electrospray mass spectrometry (ES MS) is based on two steps: the electrohydrodynamic disintegration of liquid sample solutions into small charged droplets, and solvent evaporation from charged droplets leading to the formation of desolvated ions. Both steps are performed under atmospheric pressure conditions.

Electrohydrodynamic disintegration of liquids

The electrohydrodynamic disintegration of liquids is achieved by passing a solution through a capillary and applying a high voltage to the end of the capillary. We have investigated the electrohydrodynamic processes at the end of the capillary by optical microscopy and video recording for a number of solvents and solvent mixtures. The objective of this work is to elucidate the role of various parameters such as capillary potential, flow rate of the liquid, and physical properties of the liquid such as viscosity, surface tension and conductivity of the liquid on the disintegration processes and thereby on the formation of mass spectral ions.

The experiments revealed the occurrence of several distinctly different spray modes i.e. axial spray modes, rim emission modes by which the spraying proceeds from the rim of the capillary, and droplet ejection modes. In addition, hopping between axial spraying and droplet ejection is observed and also chaotic spray modes without any observable regularity. At lower potentials near the onset of spraying, axial spray modes in a pulsed or continuous manner were observed for all solvents examined so far. However, at higher potentials the disintegration behavior differs between pure water and aqueous electrolyte solutions on the one hand and other solvents applied in ES MS such as pure methanol, water/methanol and water/acetonitrile solutions on the other hand. For water (and glycerol) the axial spray mode changes to a droplet ejection mode at potentials above the onset of a corona discharge while for the other solvents the change from the axial spray mode to a rim emission mode already occurs at low potentials below or close to the onset of a corona discharge.

Axial spray modes and rim emission modes provide conditions for the generation of sufficiently small droplets to obtain mass spectral ions by vaporization of the droplets. Spraying from the rim leads to the formation of smaller droplets than axial spraying for the same liquid. The mean size of droplets obtained in a spray mode decreases with increasing potential, with decreasing flow rate and increasing conductivity of the liquid.

The electrohydrodynamic processes leading to a change from an axial to a rim emission mode of spraying have not been sufficiently understood yet. Experiments point to a dependence of the rim emission mode on a low surface tension of the liquid and wetting of the walls. The switching from an axial spray mode to the droplet ejection mode is favored by a high field gradient at the end of the capillary, a high surface tension of the liquid and a high flow rate of the liquid, and can be qualitatively understood.

Considering the ion emission in field desorption mass spectrometry, electrohydrodynamic mass spectrometry and in liquid metal ion sources where the mass spectral ions are emitted from liquid transient protrusions, the conclusion can be drawn that in addition to the small highly charged droplets also large droplets of low charge state are formed in the electrohydrodynamic disintegration of liquids in ES MS. The formation of these droplets from a liquid jet in ES should be driven by surface tension and capillary waves rather than by field stress. The missing of molecular ions of low charge state in ES MS of biopolymers may be attributed to the formation of such large droplets which do not release mass spectral ions by subsequent solvent evaporation under ES MS conditions in the gas phase.
DESOVLATION OF IONS FROM CHARGED DROPLETS

The solvent evaporation from droplets depends on heat transfer to the droplets from the ambient gas, the extent of free solvent evaporation and the residence time of droplets in the gas. The charge state of droplets gives rise to frictional heating, a continuous renewal of the atmosphere around the droplet and a short residence time in the high pressure gas.

The field strength on the droplet surface grows by solvent evaporation. If it exceeds the Rayleigh limit the droplet decompose into several smaller droplets. (Ion evaporation cannot occur because the field strength for ion evaporation can never be smaller than the field strength required for electrohydrodynamic disintegration of charged droplets. However, analyte molecular ions with a solvation sphere might be formed by electrohydrodynamic disintegration processes under particular conditions.)

Almost complete solvent evaporation from charged droplets leads to charge condensation onto analyte molecules, a mechanism already considered by M. Dole et al. (see for example J. Chem. Phys. 49(1968), 2240). The excess charges are removed by solvent molecules due to the coulomb repulsion between the charges. Ions of opposite sign recombine and are thermally removed in case of volatile electrolytes or nonvolatile electrolytes at low concentrations. Coulomb repulsion should also lead to a declustering of small aggregates of molecular ions left by solvent evaporation from droplets. The dependence of the declustering of molecular ions on the charge state of molecules may account in part for the discrimination of molecular ions of low charge state in ES MS. Fig. 1 shows schematically the mechanism of desolvation of ions from charged droplets basically already described by M. Dole. The steps "charge condensation" and "declustering by coulomb repulsion" are not consecutive steps.

**Fig. 1:** Proposed mechanism of desolvation of ions from charged droplets in ES MS.
Control of the initial droplet size and charge in electrosprays, and ion production

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Following the recent discovery by Fenn's group of highly charged intact macromolecular ions from electrospray sources, the basic phenomena leading from the original ion in solution to a free ion are receiving increased attention (Fenn et al 1989). The steps behind electrospray ionization (ESI) are (a) formation of a Taylor cone at the tip of an electrified capillary needle through a process partly explained by Taylor (1964); (b) ejection of a steady microjet from the cone tip mostly through unknown mechanisms; (c) rupture of the jet into droplets by capillary instability; (d) evaporation of the droplets; (e) Coulomb explosions with formation of substantially smaller highly charged daughter droplets according to largely unknown fragmentation patterns; (f) emission of ions from sufficiently small droplets, mostly a mystery. The purpose of this communication is to address mechanisms (a) to (e) in order to alleviate present difficulties to electrospray liquids with high or low electrical conductivities or with high surface tension or viscosity, or at flow rates too high or too low. The advantages of controlling droplet size follow from the fact that the required residence time for evaporation in a desolvation chamber is a strong function of initial size, so that an active drying procedure is generally necessary to extract solute ions from ES droplets 3-10μm in diameter, while hardly any forced drying is required for droplets 0.3-1μm in diameter or smaller. Also, ion conversion efficiency must depend strongly on initial droplet size because most of the ion emission is from the smallest droplets while the analyte remaining in the parent droplets is largely lost. Recent work by Gomez and Tang (1991) has shown that primary droplets may be highly monodisperse, while Coulombic explosions lead to the formation of a secondary Taylor jet on the parent droplet with emission of a secondary spray of daughter droplets, also monodisperse and well over an order of magnitude smaller than the parent. Gomez and Tang also confirm earlier work by Jones and Thong (1971) reporting that electrosprayed droplets initially 100Å in diameter have a charge q near the Rayleigh limit charge qR, but the ratio q/qR decays with decreasing initial droplet diameter dP (as VdP in the range down to 40μm according to Jones and Thong; Gomez and Tang (1991b) report an average q/qR of 35% for methanol droplets 2.9μm in diameter), so that droplets originally 1μm in diameter do evaporate substantially before undergoing a first explosion. Our evidence confirms these trends for submicron ES droplets from mixtures of volatile and unvolatile liquids, which do not explode even after evaporating 90% of their initial volume. Accordingly, the daughter droplets formed in the first stage of Coulombic fragmentation may have diameters smaller than 100Å, small enough to be efficient ion emitters. This picture is in agreement with the observations from Alexandrov's group (private communication, July 1990), showing that, beyond a well defined threshold value for the droplet evaporation time between the source and the mass spectrometer, all ions suddenly appeared in the mass spectrum, while none was present below the threshold. Tuning the initial droplet size and charge would thus lead to monodisperse distributions of ion-emitting droplets, and consequently, to controlled fields at their surface and some selectivity on ion formation and charge multiplicity.

The early work of Zeleny (1914, 1917, etc.), where several prejudices prominently present in today's literature were clearly dismissed is very worth studying. For instance, he traced the difficulty to spray high surface tension liquids to the fact that the large field required to form the cone would first lead to, a discharge in the gas. He thus showed how to spray water into CO2 and even into air from sufficiently large capillary needles (1920). He also proved that highly conducting electrolytes can be easily sprayed. Smith (1986) has illustrated further some of these important practical points.

A problem to which we have recently brought some light is the elucidation of the scaling laws for the diameter of the droplets initially electrosprayed (Fernández de la Mora et al, 1990)

\[ d_P \sim (\rho U^2 y)^{1/3} \]

where \( \rho \), \( \gamma \) and \( Q \) are the liquid density, surface tension and flow rate. Eq. (1) states that the dynamic pressure of the microjet \( U^2/2 \) is comparable with the capillary pressure \( 4\gamma d_P \). Within a factor of 2, it agrees with all available ES data in the so-called rainbow mode (including those of Jones and Thong, Gomez and Tang, Kim and Turnbull and our own, mostly unpublished) in the wide range 0.03μm<dP<100μm. Preliminary measurements with glycerol droplets 0.1 μm in diameter are given by Fernández de la Mora et al (1990) together with a description of the experimental technique for sizing submicron droplets. The approximate validity of equation (1) in this size range has been
confirmed further since then by measuring droplet size distributions with two novel kinds of inertial impactors on a variety of combinations of volatile with unvolatile substances.

Equation (1) indicates that the diameter of the ES droplets can be controlled just by varying Q. However, we find that a stable spray can only be obtained within a finite interval \([Q_{\text{min}}, Q_{\text{max}}]\), which depends greatly on liquid properties and needle potential. Little is known quantitatively on how this stable region scales with the parameters of the problem. However, the qualitative description of Smith (1986) proves that increasing liquid conductivity decreases \(Q_{\text{min}}\) leading to smaller droplets. Figure 1 shows the first published report on the lower stability region in flow rate Q vs. needle voltage V space, which we have recently measured in 19% (mass) NaI-glycerol.

![Graph showing region of stable spray behavior for 19% (mass) NaI-glycerol.](image)

Fig. 1: Region of stable spray behavior for 19% (mass) NaI-glycerol (the stable region is between the two curves)

Perhaps the perspective of operating at flow rates one or two orders of magnitude smaller than current ESI standards in order to achieve initial droplet sizes in the 0.3\(\mu\)m range will attract few mass spectrometrists. Yet, the corresponding current of analyte sprayed would not decrease, but would rather increase. Indeed, reducing droplet size requires using liquids with higher conductivities, which tend to emit larger currents even at lower flow rates. An extreme illustration of this point comes from the behavior of concentrated sulfuric acid. The corresponding spray currents in CO\(_2\) are in the range of 1\(\mu\)A (well above usual ES standards), with flow rates smaller than 10\(^{-7}\)cc/s (far below established ES practice). The droplets measured in this case are in the range of 300\(\mu\)m.

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References

Gomez, S. and Tang, T. (1990a,b) ICLASS-91 meeting on atomization; (a) ES of heptane; (b) ES of methanol.
ARE THEY DESORBED OR ARE THEY DESERTED - HOW DO ES IONS FORM?

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Two quite different models have been invoked to explain the formation of gas phase ions from charged liquid droplets. According to the Charged Residue Model (CRM), originally proposed by Malcolm Dole and company, solvent evaporates from a droplet containing a single solute molecule that retains some of the charge as the last of the solvent evaporates. (1) In the Ion Desorption Model (IDM) proposed some time later by Iribarne and Thomson, a solute ion evaporates or desorbs from a droplet, alone or in aggregation with one or more molecules of solvent or solute. (2) Reflection on a variety of experimental observations persuades us that in almost all cases the IDM provides a more acceptable explanation for ion formation. In weighing these observations, some of which will be briefly described, one should remember that to account for each ion species that is identified, the CRM model assumes the existence of antecedent droplets that contain a single solute molecule of that species and the number of charges that are found on its ion. If antecedent droplets contain more than one solute molecule, or a different number of charges, the model requires an elaboration to explain how one of the plurality of solute molecules acquires its observed number of charges.

1. ES dispersion of a solution containing 0.1 g/L of arginine in 1:1 methanol:water gives rise to a mixture of singly charged ions comprising clusters of one to eight arginine molecules each with one proton attached. The same solution with 2% acetic acid added produces only monomeric ions.

2. Typical of the now familiar ESMS spectra of biopolymers alpha amylase shows peaks for ions with each of every possible number of charges from no less than 35 to no more than 58.

3. ES ions of molecules with molecular weight (M) under 800 or so are almost always singly charged.

4. Cyclosporin A, a cyclic peptide of M = 1202, gives rise to both singly and doubly charged ES ions, the former always in the majority. With gramicidin S, a cyclic peptide of M = 1142, the doubly charged ions predominate.

5. ES ions of cytochrome c (M = 12,400) from a solution containing 135 umols/L have from 12 to 21 charges. Ions from a much more dilute solution (0.35 umols/L) of the larger protein Lysozyme (M = 14,300) have from 8 to 13 ions! Moreover, small changes in the pH of cytochrome c solutions make large changes in the charge distribution on its ES ions. (4)

6. ES ions of poly(ethylene oxide) (PEO) oligomers show decreasing charge per unit length as M goes from 400 to 20,000, always less than the maximum charge predicted by a previously described model. (3) As M goes higher the charge/length ratio increases, reaching at M = 5,000,000 the maximum value allowed by the model! Not so coincidentally, the initially formed ES droplets contain but one molecule of these largest oligomers.
Even the most contrived sequence of events seems unable to produce arrays of charged droplets that could reasonably give rise by the CRM to populations of ions with features as described in these six observations. On the other hand, the IDM can provide a plausible explanation in each case:

1. Acetic acid increases the solubility of arginine so clusters cannot precipitate and form ions by the IDM. But each droplet should contain the same amount of arginine, with or without acid, and according to the CRM, produce the same ions.

2. The IDM requires only that the number of charges found on the amylose ions relate to the droplet’s surface charge density when desorption occurs. The CRM requires a remarkable distribution of droplets having only one solute molecule but every possible number of charges from 35 to 58.

3. The evidence is mounting that ES droplets always have multiple charges, no matter how small, and that daughter droplets from a Coulomb explosion always have higher charge/mass ratios than did the parent. It thus becomes difficult to envision a mechanism that could produce the singly charged droplets that the CRM requires for formation of singly charged ions.

4. Liquid chromatography experiments show cyclosporin A to be substantially more hydrophobic than gramicidin S. Thus, its ions should need less charge to escape the droplet.

5. As in the case of cyclosporin and gramicidin, the IDM would require only that lysozyme be substantially more hydrophobic than cytochrome c in order for its ions to be lifted from the droplet with fewer charges. The CRM would require an amazingly complex array of droplet charges to produce the observed distribution of charge states for these two species. Moreover, it should produce some ions with fewer charges and cannot account for the strong dependence of charge state on pH.

6. Independent experimental evidence shows that the fugacity or escaping tendency of PEO from solutions increases as \( M \) rises to 20,000 or so, remaining unchanged as \( M \) goes higher. The IDM would thus predict that ions would require decreasing charge/mass as \( M \) rises to 20,000. Viscosity increases rapidly as \( M \) goes beyond that value, slowing down ion motion and delaying desorption. Meanwhile, evaporation continues to increase surface charge density so that ions increase their charge/mass as viscosity delays their desorption.

In sum, the CRM seems much less able than the IDM to account for these and other observations on the charge state of ES ions. We conclude that the IDM embodies the mechanism that is applicable in the formation of most ES ions. The CRM can apply in the ES ionization of very large molecules.

References

4. S. K. Chowdhury, V. Katta, B. T. Chait, JACS in press.
MOLECULAR ION PRODUCTION FROM CHARGED LIQUID DROPLETS
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This paper focuses on the mechanisms involved in producing gas-phase molecular ions from charged liquid droplets at atmospheric pressure and on the problems in sampling such ions into the vacuum of the mass spectrometer. All of the spray ionization techniques such as electrospray, thermospray, and ionspray are initiated by nebulization of a liquid to produce charged liquid droplets. The processes involved in charged droplet production may vary substantially among these various spray ionization techniques, but it appears that the mechanisms involved in producing molecular ions are essentially identical in all cases.

The mechanism by which molecular ions are released from charged liquid droplets has been somewhat controversial. Thomson and Iribarne originally proposed the ion evaporation model [1] which gave a plausible picture of ion production by field assisted ion evaporation. In this model ion evaporation occurs when the field strength at the surface of the droplet, which is proportional to the droplet charge divided by the square of its radius, reaches some critical value due to vaporization of the charged droplet. Roellgen and coworkers have questioned the validity of this model [2] and have proposed instead that disintegration of the charged droplets occurs via the Rayleigh jet mechanism which eventually leads to small charged micro-droplets some of which contain only one nonvolatile sample molecule. Complete vaporization of the micro-droplets then produces the bare molecular ions which are observed in the mass spectrometer.

There is an abundance of experimental data which shows conclusively that the major mechanism for the disintegration of large charged droplets (ca. 10-100 μm) is via Rayleigh jets which produce a large number of small charged droplets, but little data has been available for droplets with diameters of 1 μm and below. Recently Zarrin [3] has determined the size distribution of aerosols produced by electrospray. These experiments employed a TSI Model 3932 Mobility Particle Sizer (TSI Inc., St. Paul, MN) which measures size distributions of aerosols in the 0.01 to 1 μm range by electrical mobility using particles with known charge distributions. A solution containing known concentrations of nonvolatile solute is electrosprayed, and after solvent evaporation the diameters of the residual dry particles are determined. The initial droplet diameter, D₀, is related to the measured solid particle diameter, D_r, by

\[ D_r = D_0 \left( \frac{C}{\rho} \right)^{-1/3} \]  

where C is the concentration of the solution (g/mL) and \( \rho \) is the density of the solid particle. Results of three such measurements employing different concentrations of nonvolatile solutes in 50:50 methanol:water electrosprayed at 0.7 μL/min are summarized in Figure 1. Under these conditions the three experiments are in good agreement and suggest that almost all of initial electrosprayed droplets have diameters between 1.0 and 1.2 μm. In addition, at the highest solid concentration residual dry particles are detected corresponding to micro-droplets with diameters less than 0.2 μm.

Our present understanding of the important mechanisms involved in production of molecular ions from charged droplets can be summarized by reference to Figure 2, where droplet charge is plotted vs droplet radius on a log-log plot. The line with a slope of 2 corresponds to a critical field
strength of $8 \times 10^8$ V/m as determined in the experiments of Katta [4], and the line with a slope of 3/2 corresponds to the Rayleigh limit. These two limits intersect in the vicinity of a particle radius of 0.05 μm and a charge of about 1000 elemental charges with the precise intersection depending on the solvent composition and the temperature. From the work of Zarrin [1] it is now clear, at least at low flow rates, that electrospray with currents in the 100 nA range produces droplets charged very close to their Rayleigh limit. As these primary droplets vaporize, they become unstable and produce a large number of micro-droplets of radius less than 0.1 μm. The final determination of which mechanism is involved in the production of molecular ions from the micro-droplets depends on the concentration of the solute and the relative affinity of of the solute and other species present for the available charge.

1. Fahim Zarrin, TSI, Inc., Particle Instruments Group, 500 Cardigan Road, P. O. Box6494, St. Paul, MN 55164., private communication.

![Figure 1. Initial droplet size distribution determined from electrospray of 50:50 methanol:water at 0.7 μL/min and spray current of 100 nA.](image1.png)

![Figure 2. Droplet charge as a function of radius illustrating paths by which charge is released by a vaporizing droplet.](image2.png)
Electrospray ionization is a gentle method of ionization that produces multiply charged gas phase ions from proteins in solution. A typical positive ion electrospray spectrum of a protein consists of a distribution of peaks, in which the different peaks in the distribution correspond to the intact protein molecule containing different numbers of attached protons.

In the present paper, we present the results of a series of investigations designed to gain an understanding of the mechanism of ionization in electrospray ionization and the factors that control the degree and extent of ionization. We discuss:

1) The relative ionization efficiencies of the various amino acid side-group functionalities, as determined from measurements made on a series of amino-terminally blocked amino acids and model peptides. These studies demonstrated that peptide residues other than Lys, Arg, and His are protonated during electrospray ionization and a scale of relative gas phase basicities was established:

   Arg > Lys > His > Trp ~ Pro > Gin

2) Effects on the observed extent of protein ionization of parameters such as solvent composition and pH. These studies showed that the extent of ionization observed in the gas phase reflects, to some degree, the equilibrium ionization of the protein in the electrospray solution. In particular, the 3-dimensional protein conformation in solution (which can be profoundly effected by the solvent composition and pH) was found to have a large effect on the degree of multiple protonation observed in the gas phase (1-4).

3) The role of the proximity of the ionizable groups and Coulomb repulsion in the gas phase on the observed extent of ionization. A method for predicting the most probable number of charges that remain attached to a peptide was developed. The method involves a calculation of the changes in enthalpy that occur when protons are removed from the ionized protein in the gas phase by residual water molecules. A comparison was made between the predictions of the theory and the observed protonation states of 21 different peptides. Good agreement was found.

In summary, we found that the observed electrospray ionization reflected the original ionization of proteins in the electrospray solutions modified by subsequent deprotonation reactions in the gas phase that are driven by Coulomb repulsion forces and collisional activation.

REFERENCES


4. V. Katta & B.T. Chait "Observation of the Heme-globin Complex in Native Myoglobin by Electrospray Ionization Mass Spectrometry" Submitted for Publication
Linear Response and Non-Constant Frequency Resolution in Stored Waveform Inverse Fourier Transform Excitation for Fourier Transform Mass Spectrometry

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FTMS excitation methods play a central role in implementing tandem MS experiments. It is important to be able to produce selective waveforms for ion isolation and to control ion kinetic energy with excitation pulses. These capabilities can be provided by the stored waveform inverse Fourier transform (SWIFT) excitation method [1].

The SWIFT method relies on the fact that ion cyclotron resonance is essentially a linear system which has been experimentally demonstrated [2]. The understanding on response of FTMS excitation has largely been based on the single-frequency or "burst" model which is invalid in cases of SWIFT or frequency sweeping excitation. In this work, a linear relation (see Eq. 1) between the voltage density and the corresponding ion radius is derived following the linear response theorem on ion motion based on the assumption of uniform excitation field. This relation is important for tandem mass spectrometric experiments performed on a FTMS spectrometer because the ion radius is closely connected to its kinetic energy. The linear relation between excitation voltage spectrum and the corresponding ion transverse velocity is helpful for understanding multi-pulse experiments without explicitly solving the Lorentz equations.

\[ r(\omega) = \frac{F(\omega)}{B_0d} \]  

(1)

Here \( r(\omega) \) is ion radius, \( F(\omega) \) is the excitation voltage density or magnitude spectrum, \( B_0 \) is the magnetic field, and \( d \) is the spacing between the excitation plates.

In this work, some of the features of the magnitude smoothing procedure [3] is also demonstrated. Excitation with constant mass resolution can be realized by using a smoothing filter with non-constant frequency bandwidth. An example for generating optimal notch excitation is given (see the Fig.).

References
4. S. Guan JAAMS in press.
Notch Excitation

(a) Specified Magnitude Spectrum

(b) Smoothed Magnitude Spectrum

(c) Waveform

("True Magnitude Spectrum" after Zero-Fill and FFT of (c))
Reduction of Space-Charge Effects In FTMS by SWIFT Excitation

Zhenmin Liang and Joseph E. Campana. EXTREL FTMS, 6416 Schroeder Road, Madison, Wl 53711.

Stored waveform Inverse Fourier transform (SWIFT) excitation provides greater flexibility and experimental versatility for Fourier transform mass spectrometry (FTMS). Space-charge effects can reduce the dynamic range in ion trapping mass spectrometry. Space-charge effects also cause a shift in ion cyclotron frequencies, which in turn limits accurate mass determination. In addition, space-charge effects deteriorate mass resolution. By using Stored Waveform Inverse Fourier Transform (SWIFT) excitation, space-charge effects can be reduced/eliminated.

I. Dynamic Range Improvement.

Our first objective of SWIFT excitation is to improve the dynamic range. High-abundance ions and low-abundance ions can be excited and detected separately by using SWIFT, and the two spectra can then be combined into one spectrum. For detection of low-abundance ions, a mass-selective waveform is constructed to eject only the major abundance ions. The low-abundance ions can then be excited and detected without the space-charge interference from the high-abundance ions. Adding the time-domain signals of the high and the low-abundance ions and then Fourier transforming the summed time-domain signal, a single mass spectrum with a larger dynamic range than a conventionally-acquired FTMS mass spectrum is obtained, as illustrated in Figure 1.

II. Reduction of Frequency Shift due to Space-Charge.

A similar scheme as described above can also be used to reduce the frequency shift caused by space-charge effects. A full-range mass spectrum can be broken into successive segments. Each segment of the mass spectrum can be detected separately without the presence of ions from other segments of the mass spectrum, and therefore without the space-charge interference from those ions. A low-pass and a high-pass filter were used to pass the signal and noise of the detected region to avoid multiple accumulation of noise. Again, the full-range mass spectrum can be reconstructed by combining the time-domain signals and by taking the Fourier transform of the summed time-domain transient signal. This method improves accurate mass measurement. An example is given Table 1.

III. High-Resolution Mass Spectrum over a Wide Mass Range.

With frequency sweep excitation, high-resolution mass spectral measurements in FTMS are subjected to two major interferences, namely folded-back peaks and space-charge broadening.

SWIFT excitation provides a solution to the above two limitations. The high definition of excite power permits selective excitation, which eliminates the folded-back peaks. SWIFT also allows highly selective ejection of ions outside the mass range of interest, which not only avoids the fold-back peaks, but also eliminates space-charge broadening. Two example are given in Figures 2 and 3.

In Figure 2, the doublet of bromochlorobenzene at m/z 192 is shown. This doublet requires a resolving power 210,000 to be separated at half height (400,000 at 10% valley). Even after ejecting all other ions except those of molecular ions (m/z of 190, 192, 194), it is difficult to separate the m/z 192 doublet. When m/z 190 is ejected, the doublet can be resolved easily at a resolving power of > 900,000.

In Figure 3 the spectra illustrate a potential application high-resolution mass measurement over the full mass range consists consists of a large number of ion peaks. First, a wide mass range spectrum is obtained to identify the number of ion peaks and their abundance in the mass spectrum. Depending on their abundance, the full spectrum can be broken into k segments where each segment contains roughly 30 m/z units. k SWIFT waveforms are constructed; each of which ejects all other ions except the segment to be detected. The resulting segments of high-resolution spectra may be reconstructed by computer to yield a single high-resolution mass spectrum for the whole mass range.

These experiments are unique to SWIFT excitation because they require the excitation of arbitrary mass ranges at high mass selectivity.

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Figure 1
Right: PFTBA Spectrum normalized to the peak at m/z 576. The spectrum was obtained by summing the transient of the low-abundance and high-abundance ions. The low-abundance ion data was acquired after ejecting high-abundance ions.
Left: PFTBA spectrum acquired by a single full range scan, normalized to the same absolute abundance as that of the right spectrum.

Table 1

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<th>ACTUAL</th>
<th>CONVENTIONAL measured (ppm)</th>
<th>SEGMENT SUMMING measured (ppm)</th>
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<td>Avg Absolute Deviation</td>
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</table>

Bromochlorobenzene, Doublet at m/z 192

Figure 2

Figure 3
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APPLICATION OF THE RF-ONLY-MODE EVENT TO HIGH PRESSURE ION CHEMISTRY IN FTMS

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Radial diffusion constrains FTMS by limiting the pressures at which the trap can effectively store ions. Cooling of an ion's internal degrees of freedom by using a bath gas is often not possible. Dynamic traps such as the QUISTOR trap ions at higher pressures with no difficulty and can be used for this purpose. We have proposed to capture this advantage for FTMS by developing a temporary dynamical trapping mode event for use in the FTMS sequence. The RF-only-mode event would be used for collisional stabilization of reaction intermediates and products.

The RF-only-mode event was implemented by modulating the trap plates of a 1.0 V, 0.0254 m cubic trap at 1.2 T with a 1000 V, 1.1 MHz sinewave during the reaction delay interval of the sequence. The waveform was turned on and off with a time constant that was changeable from 30 μs to 3 ms. During this interval, the cell could be pressurized with He to about 10^{-3} to 10^{-2} Torr by operating a Varian variable leak valve model 951-5100 with a solenoid for 1.3 s.

The transition to and from the dynamic trapping mode without He present is fairly efficient for the benzene molecular ion as indicated by at most a 20% drop in peak amplitude when compared with the same experiment but without the modulation. The RF was on for 50 ms and the benzene pressure was 1 x 10^{-7} Torr. This was true for transitions between the RF-only-mode and normal operation that occurred with time constants of 500 μs or less with modulation amplitudes ranging from 300 to 1000 V. The best result at 1000 V was a peak amplitude drop of only 6 percent, which occurred with transition time constants near 500 μs. For the longest mode transition time constant of 4 ms, the biggest drop of 30 percent occurred. For longer RF on times, the peak amplitude drops until it is 40 percent at 11 s.

Peak amplitudes first appear when the RF is turned off 0.3 s after the leak valve is closed. This was when He was pulsed to 1.4 x 10^{-3} Torr. The signal increased and maximized at 85 percent when the RF was turned off at 2.3 s after the valve closing.

Formation of the adduct CyH2O at m/z 112 was observed from the reaction of the 1,3-butadiene neutral and the vinyl methyl ether cation radical as shown in Fig. 1. 1,3-butadiene and vinyl methyl ether were introduced at pressures of 1.6 x 10^{-7} and 3.7 x 10^{-7} Torr, respectively. The He was pulsed to 3.3 x 10^{-3} Torr. The adduct peak amplitude is about 5 percent of the adduct fragment peak amplitude at m/z 80. In this experiment, the m/z 58 ion was selected by double resonance before the RF-only-mode event from ions generated with 15 eV electrons.

Normally, this adduct is not observed in FTMS and requires collisional stabilization in CI source for observation. This was observed in the same experiment as the last one; but, this one was without the RF modulation and the He pulse. There was no detected signal at m/z 112, as shown in Fig. 2. The m/z 80 peak height in Fig. 2 is 1.31 times the corresponding peak in Fig. 1.

Acknowledgments

This work was supported by the National Science Foundation Grant CHE-9017250.

References

Figure 1. RF on and He gas pulsed during the reaction delay.

Figure 2. No RF or He gas during the reaction delay.
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CHARGE EXCHANGE AND TRANSITION METAL ION REACTIONS WITH LASER DESORBED PEPTIDE NEUTRALs

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INTRODUCTION

We are currently investigating both charge exchange and transition metal ion reactions with laser desorbed peptide neutrals. We have previously demonstrated the chemical ionization of laser desorbed peptide neutrals utilizing reactions of proton transfer reagent ions stored in the analyzer cell of a Fourier transform mass spectrometer (1). In principle, this methodology can be used to study a variety of ion-molecule reactions of peptide neutrals, such as charge exchange and dissociative metal attachment. In contrast to proton transfer reactions, fragmentation observed in charge exchange reactions is initiated from an odd electron ion rather than an even electron ion. Because these different precursor ions undergo different fragmentation mechanisms, it is quite plausible to expect that these two methods of ionization will be complementary. The reaction of transition metal ions with laser desorbed peptide neutrals is also being investigated as a method to promote unique fragmentations.

EXPERIMENTAL

The experiments were performed using a Fourier transform mass spectrometer equipped with a 1-3/4" cubic analyzer cell, a 1 Tesla electromagnet, and an IonSpec data system. The main vacuum chamber is pumped with a 330 l/s turbomolecular pump to a base pressure of 2x10^-10 Torr. The introduction of reagent gases is controlled by the use of two pulsed valves and a sapphire sealed leak valve.

Peptide samples desorbed using the 193 nm (ArF) output of an excimer laser are prepared by electrospraying a methanol solution of the peptide onto a substrate which consists of a 75 nm thick film of gold-palladium coated on a macor stub. The resulting sample coverage is 10^2-10^3 monolayers. For peptide samples desorbed using the 248 nm (KrF) output of an excimer laser, a 500 monolayer coverage of sinapinic acid, a uv absorbing compound used for matrix assisted laser desorption, is first electrosprayed onto the substrate. The peptide sample is then electrosprayed on top of the matrix material producing an analyte coverage of 500 monolayers.

Charge exchange reagent ions are generated by electron ionization of a high pressure burst of a reagent gas which is admitted through a pulsed valve. Reagent metal ions are produced by laser ablation of metal foils which reside directly below the analyzer cell, or by dissociative charge exchange of metallocene compounds with neon ions.

RESULTS AND DISCUSSION

The products of the reaction of the tripeptide val-pro-leu with laser generated Fe^+ are shown in Figure 1. The condensation of Fe^+ with the tripeptide is observed at m/z 383. The neutral losses of 28 u, and 44 u suggest both decarbonylation and decarboxylation of the peptide molecule. Other significant ions found are ([C_1+Fe]^+), m/z 268, ([Y_1+Fe]^+), m/z 284, and ([B_2+Fe]^+), m/z 254. The ions M^+, A_1, A_2, Y_2, C_2, and B_1 are believed to be formed by charge exchange reactions between electronically excited Fe^+ species and the peptide neutrals. Future experiments will be directed at generating a higher proportion of ground state metal ions.

The products of the reaction of the tripeptide val-pro-leu with laser generated Fe^+ are shown in Figure 2a. The neutral losses of 28 u, and 44 u suggest both decarbonylation and decarboxylation of the peptide molecule. Other significant ions found are ([C_1+Fe]^+), m/z 268, ([Y_1+Fe]^+), m/z 284, and ([B_2+Fe]^+), m/z 254. The ions M^+, A_1, A_2, Y_2, C_2, and B_1 are believed to be formed by charge exchange reactions between electronically excited Fe^+ species and the peptide neutrals. Future experiments will be directed at generating a higher proportion of ground state metal ions.

Products of the charge exchange reaction between the tripeptide val-pro-leu and Ar^+ are shown in Figure 1. The peptide neutrals in the experiment were desorbed from a UV absorbing matrix using the 248 nm line of an excimer laser. This technique appears to reduce the amount of sample which is desorbed thus promoting only bimolecular collisions between the peptide and the reagent species. Previous charge exchange experiments performed without matrix and using the 193 nm line of an excimer laser produce ions which are formed both by charge exchange ionization of the peptide by the reagent ions and by a reaction
between these initially formed peptide ions and other peptide neutrals, i.e. self-chemical ionization. This is apparent in Figure 2b which shows the products of the reaction between pro-leu-val and Ar\(^+\), using 193 \(nm\) desorption of the peptide from a thin metal film. The presence of an abundant protonated molecular ion in this spectrum versus the that shown in Figure 2a suggest that for these conditions, desorption produces a large burst of peptide neutrals, allowing peptide ions formed by charge exchange to react with other peptide neutrals to form a protonated ion. Using the new "matrix assisted" neutral desorption technique, we have been able to perform laser desorption/chemical ionization experiments on compounds as large as the cyclic decapeptide gramicidin S, Figure 3.

We gratefully acknowledge financial support from the National Science Foundation (CHE-9024922). This work was supported in part by a research award from the American Society for Mass Spectrometry, sponsored by Finnegan MAT. Acknowledgement is made to the donors of the Petroleum Research Fund for partial support of this work.

REFERENCES

In Fourier transform mass spectrometry, ion signals are generated from the image currents induced on the detection plates of the ion trapping cell. The amplitude of an observed ICR signal is a complex function of both the number of ions in the cyclotroning packet and the ion motion in the cell. In contrast, other mass spectrometry technologies provide a direct measurement of the number of ions eliciting the observed response. In this work, a model is developed to calculate the number of ions cyclotroning in the cell from the amplitude of an ICR signal. With this technique, FTMS sensitivity and detection limits may be expressed in terms comparable to other mass spectrometry techniques. As an example, the sensitivity (C/Vg) of an EXTREL FT/MS system is determined.

Grosshans derived an equation describing the differential charge induced on the detection plates by a point charge as a function of the position of the point charge in a cell [1]. Equation 1, adapted from Grosshans' work, describes the maximum induced differential charge, $\Delta Q_{\text{max}}/q$, by a point charge, $q$, cyclotroning with radius, $r$, in an x-y plane in the center of a cubic cell, where $r$ is a fraction of the width of the cubic cell.

$$\frac{\Delta Q_{\text{max}}}{q} = \frac{16}{\pi^2} \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} \frac{(-1)^{m+n} \sinh[r\pi k_\text{max}]}{(2m+1)(2n+1) \sinh[k_\text{max} \pi/2]}$$

where

$$k_\text{max} = \sqrt{(2m+1)^2 + (2n+1)^2}$$

$\Delta Q_{\text{max}}/q$ from Equation 1 is used to determine the charge of the modeled point that would give rise to the amplitude of an observed signal. In order to correlate the modeled $\Delta Q_{\text{max}}$ with the amplitude of an observed ion signal, the voltage response of the FT/MS detection circuitry to a charge on the detection plates was determined. The output of the FT/MS preamplifier was measured to be $1.1 \times 10^{15}$ V/C.

The model was applied to Ar$^+$ signals generated in the source cell by El. The ions were excited to a radius 1/4 the width of the cell and the signal was recorded. Ion excitation to $r=0.25$ was achieved by exciting the ions with a SWIFT™ waveform with one half the minimum power necessary to begin to radially eject the ions from the cell. The Ar$^+$ signal had an initial amplitude of $5.7 \times 10^{16}$ coulombs which, through the 0.4071 ratio calculated with Equation 1 at $r=0.25$, corresponds to a $4.4 \times 10^{15}$ coulomb point charge. The $4.4 \times 10^{15}$ charge of the modeled point is equivalent to the charge of 4400 Ar$^+$ ions.
The 4400-ion charge of the modeled point calculated from the magnitude of the observed signal is not an accurate representation of the number of cyclotroning ions generating the signal. The following two methods were used to determine the number of ions generated in the cell: When the number of ions created is small compared to the number of neutrals in the EI region, the rate of ion generation, \( N \), in ions/s, can be calculated with the simple absorbance law relationship

\[
N = L \times I \times p \times \sigma_T(E)^2
\]

where \( L \) is the beam path length in cm, \( I \) is the electron beam current in electrons/s, \( p \) is the neutral density in neutrals/cm\(^3\), and \( \sigma_T(E) \) is the total ionization cross section of the sample in ions-cm\(^2\)/neutrals-electrons as a function of electron energy, \( E \) [2]. 3.7x10\(^7\) ions/s are generated by EI with the conditions used to generate the Ar\(^+\) signals: 10.0 µA beam current, 70 eV electron energy, and 1.0x10\(^{-9}\) Torr argon sample pressure in the 5.08 cm long cubic cell. The argon ionization cross section to 70 eV electrons is 3.6x10\(^{-16}\) ions-cm\(^2\)/neutrals-electrons [2]. At this ionization rate, 183,000 ions will be generated during a 5.0 ms beam.

The ionization rate calculated with Equation 2 was verified by direct measurement of the ion current generated in the cell. To make these measurements, ions were generated in the center of the FT/MS source-side cell by a continuous electron beam. An applied -75V potential to the excitation and detection plates caused the ions to spiral outward and collide with the plates. The current generated by a steady state ion collision with the excitation and detection electrodes was measured with an electrometer, providing the ionization rate. The direct ion current measurements indicate an ionization rate of 4.0x10\(^{7}\) ± 0.5x10\(^{7}\) ions/s will be generated by the EI conditions used to generate the Ar\(^+\) signals, in good agreement with the rate calculated with Equation 2.

The amplitude of Ne\(^{20}\), Kr\(^{84}\), and Xe\(^{131}\) ion signals were also correlated to theoretical \( \Delta Q_{\text{max}} \) to determine the effective charge of the modeled point. As seen with the Ar\(^+\) signals, the equivalent charge of the point is a factor of 41 ± 3 smaller than the number of ions generated in the cell by EI. Routine calculation of the number of ions generated by EI with Equation 2 is not possible since the ionization cross section of only a few atomic and simple molecular species is known. However, the factor of 41 ± 3 can be used to convert the point charge to the real number of ions. Calculating the point charge from the amplitude of an observed signal and multiplying the charge by 41 yields the number of cyclotroning ions in the cell giving rise to any signal. The 40-fold difference between the equivalent charge of the point charge model and the number of cyclotroning ions is a result of both the non-ideal behavior of the ions in the cell and a result of approximations used in the derivation of Grosshans’ equations.

The absolute sensitivity of the FT/MS instrument was investigated by using this process. 50.0 ng of naphthalene was evaporated under EI conditions and the integrated ion signal was determined. The integrated signal corresponds to 9.2x10\(^{-10}\) coulombs of ions for the 50 ng of naphthalene, yielding a sensitivity of 1.8x10\(^{-8}\) C/ng. This is in good agreement with the sensitivities of other high-performance mass spectrometers.

1. Grosshans, P. B., Thesis Ph.D., Ohio State University, 1990
Mechanisms are investigated by which the ions formed from laser desorption are trapped for detection in the FTICR trapped ion cell. Because the desorption event typically occurs outside the trapped ion cell, the LDI/FTICR experiment is similar to that performed with external source instruments in that ions must penetrate trapping fields and then experience a loss or redirection of energy with respect to the effective trapping fields to be retained. Several mechanisms for trapping externally generated ions can be postulated although experimental validation is difficult and has for the large part not been accomplished. A variety of laser desorption experiments are common including plasma ignition of metals, infrared thermal desorption of organic materials, often in the presence of an alkali salt, and matrix assisted UV laser desorption. Each of these generates an ion population with very different kinetic energy distribution, spatial distribution, and charge density, so it is likely that different mechanisms are involved for each in the trapping of ions detected by FTICR. To be presented here are data for the two separate cases of laser plasma ignition of metals and infrared thermal desorption of organic molecules.

**IR LDI of Organic Molecules.** The application of short, high energy IR radiation (>10^7 W/cm^2 from CO_2 or Nd:YAG lasers) to a wide range of organic molecules, biomolecules, and polymers has been very successful in that abundant intact cation-attached species often result. Kistemaker and others have shown the mechanism is likely a gas phase attachment process which generates low energy product ions. The general idea is that an overlap of neutral analyte molecules and higher energy cations which can penetrate the trapping potentials will react in the cell and produce a cation attachment species with sufficiently low kinetic energy to be retained. Shown in Figure 1 is a series of LDI/FTICR spectra acquired with our probe mounted fiber optic interface. Identical desorption conditions were maintained as the sample probe, coated with a 1:1 mixture of dilaurylthiodipropionate (DLTDP) and KBr, was displaced over a 40 cm distance from the trapped ion cell along the z-axis centerline. The continuous decrease in (M+K)^+ with increasing probe displacement is indicative of evolving gas phase distributions of cations and neutrals which no longer overlap in the cell. Low energy product ions formed outside the cell would have insufficient kinetic energy to penetrate the trapping electric field at the front trap plate. Also of interest is the precursor ion for this attachment. Through a series of TOF, variable trap potential, and ejection studies, we have found that the precursor is an adduct of the salt (K_2Br^+ for example) rather than the bare cation (K^+) which is responsible for formation of the product ions. Proof is shown in Figure 2 in the form of two LDI/FTICR spectra acquired for a mixture of KCl and DLTDP. Assuming the attachment process occurs in the trapped ion cell, then continuous ejection of the precursor ion will preclude formation of the (M+K)^+. In Figure 2a, K^+ is ejected, but a strong (M+K)^+ is observed. In contrast, in Figure 2b continuous ejection at the resonance corresponding to K_2Br^+ inhibits (M+K)^+ formation. This type of experiment was repeated for many salts and organic species and it was found that in general, A_2X^+ and A_3X_2^+ (A is Na, K, Rb and X is F, Cl, Br, I) are the precursors of a gas phase reaction that occurs for LDI/FTICR in the trapped ion cell to produce (M+A)^+.

**Trapping of Metal Ions from LDI.** It is a common practice to produce metal ions for ion/molecule reaction studies by using high power pulsed IR radiation to form M^+ in abundance in the trapped ion cell. Physicists have studied the characteristics of this type of ion formation for years and observed some remarkable features including the generation of multiply charged species (M^+, M^{++}, M^{3+}, M^{4+}) all with kinetic energies extending to hundreds and thousands of eV. Our interest with respect to FTICR is how such high energy ions are observed by FTMS given trap potentials which are orders of magnitude below the average energy of most ions in the distribution. Shown in Figure 3 are the kinetic energy profiles of the ion plasma generated from LDI of tin with the front trap plate of the FTMS cell biased at +80 and -80 V. Clearly a high energy plasma of both positive (M^+, M^{++}, ..) and negative (electrons) particles in what is essentially a neutral plasma encounters the trapped ion cell. This high energy plasma easily penetrates the trapping potentials, but it is not evident why abundant ions are retained in small trapping wells. Shown in Figure 4 is data which indicates what may be occurring. Here, the front trap plate is maintained at up to several hundred volts while the rear trap plate is held at 2V. Despite the skew in the trapping fields, large FTICR ion signals continue to be generated. It has been argued that the ions which are being trapped are those with sufficient energy to just make it past the front trap plate and then have some fraction of...
their axial kinetic energy deflected into radial motion. This would not explain however, Figure 4 data in which ions with in excess of several hundred eV would have to pass the front trap plate and lose hundreds of eV to be detected. The better explanation is that the neutral plasma provides shielding for low energy ions in the plasma (less than 2 eV) to pass the front trap plate. Then, deshielding from the continuously evolving plasma would expose these low energy ions to the trapping fields and allow them to be retained. This process would not have to be efficient - the number of ions generated by plasma ignition is much higher than observed for LDI of organic substrates.

**Figure 1**
DLTPD/KBr at Increasing Probe Displacement

- **5 cm from cell**
  - \( (M+K)^+ \)
  - \( K^+ \)

- **10 cm from cell**
  - \( (M+K)^+ \)
  - \( K^+ \)

- **15 cm from cell**
  - \( (M+K)^+ \)

- **20 cm from cell**
  - \( (M+K)^+ \)

**Figure 2**
LDI/FTICR of KCl and DLTP

- \( K_2Cl^- \) ejection

- \( K^+ \) ejection

**Figure 3**
TOF of + and - species formed by LD of Sn+

**Figure 4**
FTMS SIGNAL INTENSITY OF Au+ WITH VARIABLE FRONT TRAP POTENTIAL (REAR PLATE AT 2V)
The Positive and Negative Ion Condensation Chemistry of 
2, 4 - Cyclohexadieneone Iron Tricarbonyl ($\eta^4$-C$_6$H$_6$OFe(CO)$_3$) 
by FT-ICR Spectroscopy

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The gas-phase ion condensation chemistry\(^{(1,2)}\) of $\eta^4$-C$_6$H$_6$OFe(CO)$_3$ has 
been studied by Fourier transform ion cyclotron resonance (FT-ICR) mass 
spectroscopy.

For the positive ion chemistry, 40eV electron impact on the parent molecule 
generates 7 primary ions; Fe* (m/z56), (C$_5$H$_5$)Fe* (m/z121), C$_6$H$_6$Fe* (m/z122), 
C$_5$H$_5$Fe(CO)* (m/z150), C$_6$H$_6$OFe(CO)* (m/z178), C$_6$H$_6$OFe(CO)$_2$* (m/z206), and 
C$_6$H$_6$OFe(CO)$_3$* (m/z234). All the primary ions are chemically reactive with the 
neutral parent molecule. The condensation reaction products are typically of the 
form, ($\eta^4$-Diene)$_n$Fe$_n$(CO)$_m^+$ (n=2 or 3, m=1 to 5).

A fragmentation process\(^{(3)}\) of the parent molecule is proposed in scheme I. 
Here, we believe that the third CO loss is from the organic ring rather than metal 
bonded carbonyl. This is indicated by the relative rate constants of the primary 
ions\(^{(4,5)}\), shown in Figure 1. We can see that reaction rates increase with the 
increase of electron deficiency. For the structure, C$_6$H$_6$OFe*, the electron 
deficiency on the iron of m/z150 species would be 7 and its rate constant would be 
too small to fit the curve.

![Scheme I. Fragmentation process of $\eta^4$ - C$_6$H$_6$OFe(CO)$_3$](image)

![Figure 1. Trends of rate constants vs electron deficiency.](image)

![Figure 2. Temporal abundance of ions for C$_6$H$_6$OFe(CO)$^+$/C$_6$H$_6$OFe(CO)$_3$.](image)
As an example of the chemical processes, Figure 2 shows the kinetic behavior of CeH6OFe(CO)2+/parent molecule system. The reaction pathways for this system shown in the following are determined by the multiple resonance technique of FT-ICR. (n-4-CeH6O)3Fe3(CO)3+ (m/z534) does not undergo further condensation reaction due to electron saturation and spatial obstruction.

\[
\begin{align*}
(C_6H_6O)_3Fe(CO)_3^+ + M \rightarrow (C_6H_6O)_2Fe_2(CO)_2^+ + 3CO \\
(C_6H_6O)_2Fe_2(CO)_2^+ + 2CO \\
(C_6H_6O)_2Fe_2(CO)_3^+ + 2CO \\
(C_6H_6O)_2Fe_2(CO)_3^+ + M \\
\end{align*}
\]

For the negative chemistry, dissociative electron capture by the parent molecule of 0.5eV electrons generates two primary negative ions: C6H6OFe(CO)- (m/e178), and C6H6OFe(CO)2- (m/z206). The 17e species, C6H6OFe(CO)2- is unreactive with the neutral parent molecule. CeH6OFe(CO)- reacts with the parent to form products of the form (C6H6O)nFe(CO)m (n=2 to 4, m=1 to 6).

For the positive chemistry, products can be proposed from analysis of the reaction pathways and the reaction rate constants.

Some examples for ions generated from the primary ion C6H6OFe(CO)+ are shown in the following:

Unlike in the iron carbonyl system, only single metal-metal bonds are predicted for the structures of ionic products.

References:
The rearrangement of carbenium ions by 1,2-migration is ubiquitous in organic chemistry with a rich body of information concerning the relative migratory aptitude of various groups[1]. α-Silyl-substituted carbenium ions (R3Si-CH2+) have been implicated in rearrangements involving 1,2-migration in α-functionalized silanes in solution, however, there is virtually no information regarding the relative migratory aptitude of various groups[2,3]. This is a consequence of the instability of organosiliconium ions (R3Si+) in solution under conditions where analogous carbenium ions are long lived[4]. We have initiated studies involving 1,2-migration of α-silyl-substituted carbenium ions by using Fourier transform mass Spectrometry (FTMS).

Nascent α-silyl-substituted carbenium ions are generated from the corresponding (chloromethyl)silane by three methods; 1) protonolysis, 2) chloride abstraction by Si(CH3)4+, and 3) direct electron impact ionization. For example, protonolysis of (CH3)2HSiCH2Cl by CH5+ yields primarily (CH3)2Si-CH2+ (1), process 1. The incipient carbenium ion, 1, may subsequently rearrange by either a 1,2-hydrogen migration or by a 1,2-methyl migration, processes 2 and 3, with both migrations highly exothermic. Theory has revealed that there is essentially no barrier for the strongly exothermic (~40 kcal/mol) 1,2-hydrogen migration for H3Si-CH2+, process 4[5]. In addition, 1,2-methyl migration for (CH3)2Si-CH2+ yielding (CH3)2Si+CH(CH3) is highly exothermic (~32 kcal/mol) and has little or no barrier. These results suggest that both 1,2-hydrogen and 1,2-methyl migration are feasible for carbenium ion 1.
Distinguishing the two rearrangement channels for carbenium ion 1 requires that the two distinct silicenium ions are distinguishable. We have found that specific ion/molecule reactions can readily distinguish structural silicenium ion isomers. For example, methanol reacts with Si(CH₃)₃⁺ yielding exclusively elimination of methane whereas SiH(CH₃)(C₂H₅)⁺ yields competitive eliminations of H₂ (0.49), CH₄ (0.23) and C₂H₆ (0.28). In addition, ethene- C₂ yields elimination of the corresponding olefin for reaction of organosilicenium ions containing a 2-carbon or larger alkyl substituent process 5. For the two SiC₃H₉⁺ isomers, Si(CH₃)⁺ is inert with ethene-C₂,

\[
\text{(CH}_3)_2\text{Si(C}_\text{H}_3\text{)}_{2\text{n+1}}^+ + \text{C}_2\text{H}_4 \rightarrow \text{(CH}_3)_3\text{Si}^+ + \text{C}_2\text{H}_5\text{H}_2 \tag{5}
\]

however, SiH(CH₃)(C₂H₅)⁺ yields clean elimination of unlabeled ethene (i.e., m/z 73 goes to m/z 75 cleanly). By combining these two specific ion/molecule reaction probes, virtually any structural silicenium isomers can be distinguished.

Employing the above methods reveals that nascent carbenium ion 1 undergoes exclusive 1,2-hydrogen migration. In addition, rearrangement of the following nascent α-silyl-substituted carbenium ions was also investigated; (CH₃)₂Si-CH₂⁺ (2), (CH₃)₃Si-CH₂CH₃ (3), (CD₃)₂Si-CH₂CH₃ (3δ) and (C₂H₅)₂Si(CH₃)CH₂⁺ (5). Both 2 and 3 undergo 1,2-methyl migration yielding the corresponding silicenium ion. The isotopically labeled carbenium ion, 3δ, confirmed that the rearrangement simply involves a 1,2-methyl migration (i.e., no isotopic scrambling occurs). Structural studies reveal that the incipient carbenium ion, 4, undergoes competitive 1,2-phenyl (0.94) and 1,2-hydrogen (0.06) migration. These results suggest the following relative group migratory aptitude: Aryl > H >> Me.

A Finnigan ion trap mass spectrometer (ITMS) has been coupled with a unique mounting platform that permits the rapid and precise placement of both laser optical and ion optical elements within the vacuum system. Ion lens elements and ion trap electrodes can be accurately positioned in new configurations and the laser beam can be readily focused into the volume of the ion trap or onto a sample probe for facile implementation of both ion and laser optical experiments. Laser photodissociation experiments serve as a powerful probe of ion trajectories within the ion trap. The ring electrode of the ion trap has been modified so that the interior volume of the trap can be probed by a XeCl excimer laser (308 nm) at different axial positions along the ion trajectory. In one series of experiments, mass isolated benzoyl ion (C$_6$H$_5$CO$^+$ from acetophenone) is dissociated to C$_6$H$_5^+$ upon photoactivation at 308 nm. The abundance of the photo-produced ion at m/z 77 is then related to the number density of the parent ion at the axial position probed by the laser. Figure 1 shows the axial ion distribution within the ion trap as a function of q$_z$. Ion density near the center of the trap increases non-linearly with q$_z$ (or RF voltage) as expected. Figure 2 dramatically demonstrates the effectiveness of helium buffer gas in stabilizing ion trajectories within the ion trap. Stored ions may also be excited by application of a supplementary RF voltage at the proper frequency to the end-cap electrodes ("RF tickle"). Figure 3 shows the elongation of the trajectory of the C$_6$H$_5$CO$^-$ ion stored at q$_z = 0.4$ effected by a "tickle" by 100 mV of RF voltage (approximately 151 kHz) applied to the end-caps. Photoionization studies can also be performed to illustrate the effects of ionization at different axial positions. Figure 4 shows the variation in abundance of the molecular ion of aniline as a function of q$_z$ and axial position formed by photoionization at 308 nm. The storage efficiency of the ion trap for ions produced by photoionization is seen to decrease as q$_z$ increases and, at least in these experiments, the sensitivity of the ion trap varies little for ionization occurring at displacements of a few millimeters from the center of the ion trap.

Figure 1. Axial distribution of C$_6$H$_5$CO$^+$ as a function of q$_z$. 
Figure 2. Helium buffer gas stabilization of ion trajectories within the ion trap. Ionization times were adjusted to maintain the same signal intensity for C₆H₆CO⁺.

Figure 3. Perturbation of the trajectory of C₆H₆CO⁺ by a "tickle" by 100 mV of RF voltage applied to the end-caps.

Figure 4. The abundance of the molecular ion of aniline formed by photoionization at different axial positions and values of q₂.
A method is reported by which surface-induced dissociation (SID) is used to activate ions stored in a quadrupole ion trap mass spectrometer. The method employs a short (<5 μs), fast-rising (<200 ns rise time), high voltage (0-900 volt) dc pulse which is applied to the endcaps of a standard Paul-type quadrupole ion trap. Since the pulse rise time is considerably faster than one RF cycle (1 μs), it provides a means of effecting almost instantaneous radial instability of ions stored in the ion trap. The ions then collide with the surface of the ring electrode resulting in fragmentation. The fragments are scanned out of the ion trap using the conventional mass-selective instability scan mode. This SID method of activation is in sharp contrast to collisionally activated dissociation (CAD) where relatively long applications of a low voltage ac signal (0.1 to 2.0 volt for 10-100 ms) is used to resonantly excite the ions and cause dissociative collisions with helium.

In order to obtain more insight into ion motion in the trap under the conditions used here, ion trajectories were simulated using a program developed in our laboratory. The program has been found to give good results in comparison with experiments which measure the kinetic energies with which ions are ejected from the trap. Figures 1 and 2 display both the radial and axial positions and velocities for the pyrene molecular ion in the SID experiment described above. The radial simulation shows that approximately 2 μs after the initiation of the dc pulse the pyrene molecular ion strikes the ring electrode. The collision energies in this simulation depend heavily on the phase and position of the ion when the pulse is applied, but the typical data shown demonstrate that over 100 eV of radial translational kinetic energy is available. The simulation does not specifically include the effects of collisions with helium but at a pressure of 10⁻⁴ torr the collision frequency is on the order of one collision in ten rf cycles. Therefore, typically zero or one collision with helium occurs during the activation time. Simulation of motion in the axial direction, Figure 2, shows that the pyrene molecular ions remain in stable oscillation in this direction and that the axial kinetic energies remain small.

Sufficient internal energy is acquired in the surface collision to cause high energy fragmentations of relatively intractable molecular ions such as pyrene as is shown in Figure 3. The formation of the peak at m/z 122, although of very low intensity, requires approximately 17 eV of internal energy. The dissociations of limonene (Figure 4) are used to demonstrate that high energy demand processes increase in relative importance in the dc pulse experiment compared to the usual resonance excitation method used to cause activation. It is also apparent from the results obtained on limonene that a relatively wide range of internal energies is deposited in the activated parent ion. A small part may be due to contributions from CAD processes. However, the simulations suggest an additional interpretation, namely that collision energy and angle vary greatly with the initial (pre-dc pulse) conditions of the stored ion and hence a wide range of internal energy depositions can be expected.

The most direct evidence that surface induced dissociation is contributing to the product MS/MS spectra when the dc pulse is applied is found in data taken for the benzene molecular ion (Figure 5). Fragmentation occurs to give m/z 52, 51 and 50, among other ions. When a delay is introduced between the phase of the main rf cycle and the onset of application of the dc pulse, the abundances of these fragment ions vary with the magnitude of the delay in the fashion shown in Figure 6. Clearly, the interaction between the fields created by the dc and the main rf voltages has a strong effect on ion production. This is expected for the SID process, since the main rf field will alternately assist and resist acceleration of the parent ions to the radial electrode by the axial dc.
References


Figures 1 & 2. Simulation of motion of pyrene molecular ion in the radial (1) and axial (2) direction. Individual parts of the figure show the applied potentials, the respective positions and kinetic energy. The simulation ends when the ion strikes the ring electrode.

Figure 3. Product spectrum of pyrene molecular ion recorded after applying a 325 V dc pulse for 3.65 μs at q_z = 0.43.

Figure 4. Product spectra of the limonene molecular ion after application of a dc pulse (305 V, 2.3 μs, q_z = 0.5). Inset shows a typical CAD spectrum produced by the conventional method of resonance excitation (ac = 700 mV at 91545 Hz for 3 ms, q_z = 0.23) for the mass range 65 to 100.

Figure 5. Product spectrum of benzene molecular ion recorded after applying a 348 V dc pulse for 1.5 μs at q_z = 0.5.

Figure 6. Abundance of fragment ions generated from the benzene molecular ion as a function of the rf phase delay between an arbitrary point in the rf phase and application of a dc pulse.
High Resolution High Sensitivity Tandem Mass Spectrometry in the Ion Trap Mass Spectrometer

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Progress is reported on the long term objective of transforming the quadrupole ion trap from a simple GC/MS instrument into a high performance tandem mass spectrometer. Using slow scan speeds as suggested earlier, mass resolution for peptides has been improved from 2,500 (FWHM) at 1500 dalton to more than 100,000 (Figure 1). This facilitates the recognition of effects such as reduction accompanying desorption ionization (Figure 2). In the case of salt clusters, resolutions in excess of 10^4 have been recorded (Figure 3). The resolution increases with mass and the factors which control resolution (scan speed, instrumental stability, and ion density, \textit{inter alia}) have been delineated with the assistance of simulations of ion motion. Not only is high resolution achieved in the mass spectrum but comparable resolution is achieved in MS/MS experiments, both in parent ion selection (MSI), and in fragment ion analysis (MSII). Figure 4 illustrates that in the case of protonated substance P, each of the major isotopic forms of the molecular ion gives characteristic fragments which together account for the product MS/MS spectrum of the mixture of molecular ions. The increased information available using high resolution MS/MS is illustrated in the case of gramacidin S in Figure 5, which displays MS/MS data taken on the unresolved protonated molecule as well as data for the ^13C,-isotopic form. These experiments were typically performed on picomole amounts of material which lasted for an hour or more.


Figure 1. Resolution achieved in the molecular ion region for the peptide, substance P. The mass spectrum (nominal masses shown) was recorded using resonance ejection with a 2.25x mass range extension. The scan speed was 55.6 dalton/second and the data shown are for a single scan taken in 0.02 seconds.

Figure 2. Cs+ ion bombardment mass spectrum of somatostatin recorded using glycerol (upper) and glycerol/dithiothreitol (lower) as matrix. Partial reduction of the disulfide double bond occurs in glycerol and is complete when the threitol is added.
Figure 3. Resolution in excess of a million achieved for \((\text{CsI})_n\text{Cs}^+\) using resonant ejection with a 6x mass range extension and a 2000x reduction in scan speed.

Figure 4. MS/MS data on substance P showing product ion spectrum recorded for the unresolved MH\(^+\) ion (upper) and the same region of the spectrum for the all\(^{12}\text{C}\) and the \(^{13}\text{C}-\text{MH}^+\) ions, respectively. The expected abundance distribution of the unresolved case shown in the box, matches that recorded. The experiment was done using 74 pmole of sample although this lasted much longer than the time needed to record this data.

Figure 5. MS/MS spectrum of peptide gramacidin S. Ancillary figures show the molecular ion region under high resolution and the result of isolating the \(^{13}\text{C}-\text{MH}^+\) ion at m/z 1142. The MS/MS spectrum derived by dissociation of this selected ion is also shown.
MICROCAPILLARY HPLC-ESI TANDEM MASS SPECTROMETRY: NEW METHODOLOGY FOR PROTEIN SEQUENCE ANALYSIS.

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Fused silica columns (75μm 75 micron i.d.) are employed routinely in our laboratory for microcapillary HPLC separations of peptides conducted on-line with an electrospray ionization source on the Finnigan triple quadrupole mass spectrometer. Peptide mixtures to be analyzed are washed directly onto the head of the micro C_{18}-column and the individual components are then eluted into the triple quadrupole mass spectrometer for sequence analysis with a 15 min gradient of 0-80% acetonitrile at flow rates of 1-2 ul/min. Described here is the use of the above microcapillary HPLC columns to characterize posttranslational modifications of Class III, β-tubulin in microtubules and to identify the regulatory phosphorylation sites on mitogen activated protein kinase (MAP-kinase).

Microtubules, assembled from two similar 50 kDa proteins designated α- and β-tubulin, are involved in segregation of chromosomes during cell division, cell motility, organelle transport, and maintenance of cell shape. Class III, β-tubulin in mammalian brain cells undergoes a novel, developmentally regulated posttranslational modification, polyglutamylation plus phosphorylation on the C-terminal, 14 residue peptide having the sequence, YEDDEEESEAQGPK (1). To locate the sites of these modifications, a mixture of brain tubulins was digested the cyanogen bromide, and the C-terminal peptides from class III, β-tubulin were removed from the mixture by binding them to a monoclonal antibody, TuJ1. Bound peptides were then liberated from the antibody, converted to the corresponding methyl esters, and introduced to the mass spectrometer via the microcapillary HPLC column described above. Shown in Fig. 1 is the CAD spectrum recorded on the (M+2H)⁺⁺ ion derived from one of the posttranslationally modified peptides. Fragment ions of type y indicate that the last 12 residues of this 14 residue peptide are unmodified. Ions of type b₁ and b₂ are shifted to higher mass by 80 and 157 daltons. Accordingly, sites of phosphorylation and glutamylation are residues 1 and 2, respectively.

To characterize the posttranslational modifications of additional, less abundant, tubulin isotypes, we envisage use of a set of microcolumns connected in series. Immobilized enzyme to digest the crude tubulin mixture will be placed in the first column. The second column will contain immobilized monoclonal antibody to selectively remove peptides from a particular isotype and these will then be fractionated on a third column containing HPLC packing and eluted directly into the tandem mass spectrometer for sequence analysis.

MAP kinase, a 42 kd serine/threonine specific protein kinase of unknown sequence, becomes activated after phosphorylation on both tyrosine and threonine. Insulin, epidermal growth factor, platelet derived growth factor, and a number of mitogens all promote this activation. To deduce the phosphorylation sites on this protein, ³²P-labeled protein was isolated from mouse cell culture, treated with trypsin and the resulting peptides fractionated by microbore HPLC. One third of the fraction containing radiolabeled peptide (9 10 pmol) was then rechromatographed on the above microcapillary column and sequenced by tandem mass spectrometry (2). Shown in Fig. 2 is the CAD spectrum recorded on the (M+3H)⁴⁺⁺ ions derived from the doubly phosphorylated peptide. Assignment of charge to a particular ion in Fig. 2 was greatly facilitated by recording a second CAD spectrum on a 10 pmol sample of the corresponding peptide ethyl ester. Conversion of the peptide sample to its ethyl ester increases the mass of any particular fragment by 28, 14, and 9.3 daltons per carboxylic acid group for ions having a charge of +1, +2, and +3, respectively. Partial elimination of small neutral molecules such as ammonia, water and phosphoric acid also facilitates assignment of the charge on a particular fragment. A combination of singly, doubly, and triply charged fragments of type y were employed to deduce the complete sequence of this 19 residue peptide. Residues 13 and 15 were assigned as the sites of phosphorylation.


V A D P D H D H T G F X T - P E Y - P V A T R
TANDEM MASS SPECTROMETRIC SEQUENCING OF CYTOKINE-INDUCED CELLULAR PROTEINS ISOLATED FROM 2-D GEL ELECTROPHORESIS


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Tandem mass spectrometry continues to play a vital role in solving a variety of biological structural problems [1]. Previously, our laboratory demonstrated the feasibility of using a four sector tandem mass spectrometer with multi-channel array detection to obtain sequence information from proteins isolated from SDS-PAGE gels [2]. We now report the sequencing of cytokine-induced cellular proteins which have been isolated from two dimensional (2-D) gels at the 50 picomole level.

Tumor necrosis factor (TNF) and interferon-γ (IFN-γ) are cytokines that regulate the production or suppression of cellular proteins in normal [3] as well as tumor cells [4]. To understand the role these cytokines play in regulating cell growth it is vital that the primary structure of the proteins whose synthesis they enhance or suppress be determined as knowledge of the sequence of cytokine-induced or -suppressed cellular proteins will lead to identification of their function as well.

Proteins in lysates from cytokine treated human A375 melanoma cells were separated using 2-D gel electrophoresis employing isoelectric focusing in the first dimension followed by SDS-PAGE in the second. Considerable effort had been expended in attempts to sequence these proteins by Edman methods. In numerous instances sequence information was not obtained presumably because of blocked N-termini. Gel plugs containing three proteins whose synthesis is enhanced by IFN-γ were excised from the gel and purified by electroelution using a Centrisyn Gel Eluter followed by Konigsberg acetone precipitation (5). Of particular interest was a 50kD protein with an isoelectric point (pI) of 5.9. The purified proteins were then digested with trypsin and the resulting peptides separated using reverse phase microbore HPLC. Liquid secondary ion mass spectrometry (LSIMS), performed on a VG Analytical 70SE double focusing mass spectrometer, was used to obtain molecular ion maps of each digest. A cesium ion beam and accelerating voltage of 8kV was used for this instrument and all scans were processed on a VAX station 3100 with a OPUS data system. Collision induced dissociation (CID) mass spectra were obtained, for tryptic components which gave the most abundant molecular ions, using a Kratos Concept four sector tandem mass spectrometer equipped with a 4% multichannel array detection system.

Several sequences were obtained from interpretation of the CID data. The 50kD protein with pI 5.9 yielded three peptide sequences. A search of the Protein Identification Resource Dayhoff database revealed that two of the sequences showed 100% homology to a human cytoskeletal protein. The remaining sequence showed no homology to any of the proteins in the database. We are currently refining our protocol so that a greater quantity of these proteins can be isolated. This work has demonstrated that by combining the sensitivity of high performance tandem mass spectrometry with the superior resolving power of 2-D polyacrylamide gel electrophoresis it is now possible to isolate and obtain sequence information from an individual protein originating from an extensively heterogeneous cell lysate. In addition, our procedure is not restricted to proteins and peptides which have only unblocked N-termini.

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References:

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Figure 1. Tandem Mass Spectrum of 50kDa/pI 5.9 Tryptic Peptide SLDLDSLIAEVK, MH+ = 1302.8, Isolated From 2-D PAGE.
The Utility of N-Peracetylation of Proteins for their Structure Determination by Mass Spectrometry

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Mass spectral analysis of acetylated peptides has been shown to be useful for determining the number of lysines in the native peptide, by examining the mass shift in the molecular weight of the peptide before and after acetylation. The isobaric amino acids lysine and glutamine can be differentiated by examining the CID spectrum of the acetylated peptide. The mass of the lysine residue is shifted to 160 u while the glutamine residue remains at 128 u.

Determination of the linear sequence of an unknown peptide in our laboratory normally involves enzymatic or chemical digestion of the protein, molecular weight analysis of the hydrolysis products by FAB-MS, and then determination of their sequence using CID and MS/MS. The first digestion is performed with trypsin, because it yields reliable cleavages at the C-terminal side of arginine and lysine. The digest products usually give good CID spectra, because a basic amino acid at either the N- or C-terminus directs fragmentation.

Enzymatic digestion with other common proteases, such as chymotrypsin or Endo Glu-C, frequently results in peptides which have either no basic amino acids, or they are in the interior of the sequence. The CID spectra of these peptides are sometimes either quite complex or the sequence information is incomplete. A useful technique to overcome this problem is derivatization of the N-terminus with a quaternary ammonium group. This functional group directs the fragmentation to such an extent, that virtually only N-terminal a and d are observed, regardless of the other structural features of the sequence. Unfortunately, because this derivatization reaction involves a primary amino group, derivatization of the e amino group of lysine is a competitive process. Since the ion current is now split into two product species, the sensitivity of the analysis is reduced. Acetylation of the peptide to block the lysines is not useful, since this would also acetylate the N-terminus to which the quaternary ammonium group is being attached.

We have recently been investigating the utility of N-acetylating intact proteins, and then analyzing the CID spectra of peptides which result from enzymatic digestion. The protein (1-10 nmoles) is dissolved in glacial acetic acid and then an equal amount of acetic anhydride is added. Reactions are carried out at 40 °C for 2-3 hours. Under these conditions, the protein is usually over acetylated, therefore, after removal of the reagents by lyophilization, the protein is redissolved in 1M NH₄OH and allowed to sit at room temperature for 1 hour. This step removes most of the O-acetyl groups formed in the first step. LD-TOF analysis after the derivatization of Cytochrome C (Mₐ = 12359) reveals an average MW of 13,199 daltons, indicating the addition of 20 acetyl groups. Cytochrome C has 19 lysines and an acetylated N-terminus, i.e. the average of one hydroxyl group has been esterified.

![Figure 1. Molecular Weight Determination of Acetyl Cytochrome C by LD-TOF-MS](image-url)
For reasons discussed above, enzymatic digests other than trypsin were used to generate peptides for sequence determination by CID. Chymotryptic digestion of acetylated Cytochrome C and analysis of the resulting peptides by FAB, yielded complete coverage of the protein sequence and showed that several peptides, each of which contained one or more threonine residues, had a complementary peptide 42 u higher. Examination of the CID spectra of several of these peptides revealed that in fact the threonines had been partly O-acetylated. Only one of the over acetylated peptides was present in high abundance. Since our purpose in this work is to examine the enzymatic digestion of the acetylated proteins, and the CID spectra of the resulting peptides, we are not overly concerned with a small amount of over acetylation. A further point worth mentioning, is that because the chymotryptic digestion of cytochrome C requires over 24 hrs, a significant number of tryptic cleavages occurred in the native protein at lysine. This produced many small peptides, a significant number of which were lost, and resulted in only 64% coverage of the amino acid sequence. Since the acetylation of the lysines renders them inert to trypsin, the chymotryptic digest of acetylated cytochrome C contains only chymotryptic peptides (even after a 37 hour digestion) and is complete.

Since lysine occurs more frequently than arginine, chymotryptic and Endo Glu-C digests of acetylated proteins produce mainly peptides containing no basic amino acids. The CID spectra of such a typical peptide is shown in Figure 2a. These spectra are generally simple, showing only b, and y, series ions. The presence of acetyl lysine is easily recognized by the presence of an abundant cyclized immonium ion (KAc) at m/z 126 and an ion resulting from the loss of the acetylated side chain from the [M+H]+ ion. Unfortunately, these spectra do not contain either d, or w, side chain cleavage ions, and, therefore, as is the case in Figure 2a cannot distinguish leucine from isoleucine. Since we would normally always begin the structure determination of a protein with a tryptic digest of the native protein, the two digests should be examined for overlapping sequences. Figure 2b shows a tryptic peptide which overlaps five of the amino acids in the chymotryptic acetylated peptide. This spectrum clearly indicates that both of the amino acids in question in Figure 2a are leucine. If no overlapping peptides were found, an N-terminal quaternary ammonium derivative of the chymotryptic peptide could be made, and its CID spectrum measured.

Finally, unless the N-terminus of the protein is already blocked, it can be readily determined, since the N-terminal peptide will be the only one with an acetylated N-terminus. This is readily determined from the CID spectrum, since N-terminally acetylated peptides give abundant b, ions.
DESIGNING A REAGENT TO CLEAVE CARBONYL-NITROGEN BONDS IN PEPTIDES IN AN ANION-MOLECULE REACTION.

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The current method of obtaining protein sequence information by mass spectrometry is based on the analysis of the fragment ions which are formed in certain collisional processes (FAB, SIMS, MS/MS-CAD, etc.). Using chemical reactions to cleave peptide bond is an attractive alternative. In contrast to these collisional processes which are largely controlled by energy redistribution, the efficiency and selectivity of chemical reactions are determined by the reactivity of the substrates and reagents and therefore, can be controlled much better with the knowledge of chemical reactivity of amides, peptides and proteins. Endothermic proton transfer has been used to induce fragmentation at low collision energy with an increased efficiency [1]. Our approach is based on the use of chemical reaction to cleave the peptide bond in a specific fashion. The development of reagents to cleave carbonyl-nitrogen bond of peptides in a gas-phase anion-molecule reaction will be the subject of this report.

Amides are relatively unreactive both in solution and in the gas phase. Although, cleavage of amide bond has been observed in the reaction of HOO' with HCO-NMe₂ [2] and, in our flowing afterglow study, with MeCO-NMe₂, proton transfer becomes predominate when acidic protons are available in amides, which would be more true in peptides. We then adopted the strategy outlined in Scheme 1 where the deprotonated amides are used to react with neutral reagents to block the proton transfer. The neutral reagents are chosen based on their ability to generate nucleophilic species in the collision complex and to activate the amine leaving group. A variety of neutral reagents were studied and several of them were found to cleave the MeCO-NMe₂ bond as shown in Table 1. The most effective of them, S-ethyl trifluorothioacetate, CF₃COSEt reacts with deprotonated anions of several amides and our dipeptide model compound to give corresponding RCO-NR' bond cleavage product CF₃CONR' (Table 2) in a facile reaction. The major competing pathway with the formation of CF₃CONR' is the formation of the product ion corresponding to the loss of EtSH from the collision complex, [RCONR' + CF₃COSEt - EtSH]. The EtS' ion formed in the collision complex can attack a carbonyl group activated by CF₃CO or abstract an available proton as shown in Scheme 2, the ratios of the two products are found to correlate roughly with their basicity (Table 2).

Our results demonstrated the cleavage of RCO-NR' bond of amides and dipeptide analogs specifically and effectively in a gas phase anion-molecule reaction. This is the first step towards a viable alternative to the existing techniques of mass spectrometric analysis of protein sequence. A possible practical application involves low energy CAD experiment of [M-H]⁺ ion of a peptide with hydrogen peroxide, HOOH and trifluoroacetic acid esters, CF₃COX (X = OR or SR) to form fragment ions corresponding to N- and C-terminal sequence of the peptide respectively.

Acknowledgement
This work was supported by a NSF-PYI award to JJG.

References
Table 1. Results of MeCO–NMe$^-$ + X–Y

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<th>X–Y</th>
<th>X–NMe$^-$ (%)</th>
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<tr>
<td>CF$_3$CO–SEt</td>
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<td>(MeS)C$_6$F$_4$–F</td>
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<tr>
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<tr>
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Table 2. Results of RCO–NR$^+$ + CF$_3$CO–SEt.

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<th>RCO–NR$^+$ [RCONR$^+$+N–EtSH]</th>
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<th>yield PA kcal/mol</th>
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$^a$ Proton affinity Those in italic are estimated.

Scheme 1

Scheme 2
FACILE ASSIGNMENT OF DAUGHTER IONS PRODUCED BY COLLISION-INDUCED DECOMPOSITION OF A PEPTIDE IN FAB MASS SPECTROMETRY

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Collision-induced decomposition (CID) method combined with two- or four-sector (MS/MS) mass spectrometer is making a significant contribution to sequence analysis of a peptide and protein.1,2,3 In a CID mass spectrum, the daughter ions produced from a precursor ion provide information on the amino acid sequence of a peptide from both amino- and carboxyl-terminal. However, one of the drawbacks of CID spectra is the insufficient assignment of complicated signals of daughter ions from peptides to amino acid sequences.

The enzyme-catalyzed isotope exchange with $^{18}$O in $^{18}$O has been successfully applied to analysis of biological compounds by mass spectrometry.4,5,6 In protein sequence analysis it was found to be useful for identification of the C-terminal peptide or amino acid of a protein. In this work we report a method for the assignment of sequence ions of a peptide to the amino acid sequence by CID/linked-scan analysis in combination with the above procedure for incorporating $^{18}$O into the C-terminus of the peptide. The method is comprised of the following procedures: 1) A protein is digested by an enzyme in an appropriate buffer containing $^{18}$O-labeled water (40 atom % $^{18}$O). $^{18}$O atoms are incorporated into a part of the C-terminal α-carboxyl groups of all the digested peptides of a protein except the original C-terminal one. 2) The digest is separated into the constituent peptides by reversed-phase high-performance liquid chromatography. 3) The peptides are submitted to CID mass measurement using a B/E linked-scan mode, where the electric field (E) and magnetic field (B) were changed, keeping the ratio of B/E constant. The precursor ion of the peptide containing $^{18}$O in part of its C-terminal carboxyl group showed a doublet signal (M+1 and M+3). Thus the CID/linked-scan spectrum of the $^{18}$O-labeled peptide gave two series of sequence ions: one, originating from the N-terminus of the peptide, had the natural isotopic ion distributions, while the other, originating from the C-terminus, showed doublet signals due to partial incorporation of $^{18}$O into the C-terminal α-carboxyl group. These two series of sequence ions could be readily differentiated in a single CID/linked-scan spectrum.

Figure 1 shows a comparison of the typical CID/linked-scan mass spectra of one of the tryptic peptides derived from sperm whale myoglobin (Ala-Leu-Glu-Leu-Phe-Arg (134-139)) obtained in a buffer prepared with 40 atom % $^{18}$O (upper) and normal water (lower). The sequence ions derived from the N-terminus of both $^{18}$O-labeled and non-labeled peptides (a and b series) gave the natural isotopic ion distributions, while those from the C-terminus of $^{18}$O-labeled peptide (x, y, z and w series) in the upper spectrum gave the doublet signals due to partial incorporation of $^{18}$O into the C-terminal carboxyl group. It demonstrated that $^{18}$O atoms were retained at the α-carboxyl group of the sequence ions originating from the C-terminus of the peptide. Thus, the sequence ions could be readily classified as N-(-Glu-Leu-Phe-) and C-terminal (Ala-Leu-Glu-Leu-Phe-) ones by a single-scan spectrum of the $^{18}$O-labeled peptide and unambiguously assigned to the above amino acid sequence.

This work demonstrates that the CID/linked-scan method in combination with a procedure for incorporating $^{18}$O into the C-terminus of the peptide is effective for the analysis of sequence ions of a peptide. It allows reliable assignment of sequence ions to amino acid sequence with only small samples, and can be
readily applied to a conventional two-sector mass spectrometer equipped with a collision cell.

Acknowledgment
We wish to thank Mr. Y. Kammei (JEOL Co.) for his helpful discussion.

References

Figure 1. CID/linkccl-scan spectra of a tryptic peptide (Ala-Leu-Glu-Leu-Phe-Arg (134-139)) of sperm whale myoglobin obtained in buffer prepared with 40 atom % H$_2$O (upper) and normal water (lower). The sequence ions are denoted according to the previous paper. The solid and broken lines in the upper spectrum show a series of sequence ions containing N- and C-terminus of the peptide, respectively.
Assigning binding sites of a peptide to a transition metal ion is of significant biological importance. Studies of interactions between peptides and transition metal ions have been carried out in our group by \(^{252}\text{Cf}\) plasma desorption mass spectrometry (PDMS). Generally speaking, potential binding sites of a peptide to a metal ion are the N-terminal amino group, the C-terminal carboxyl group, the deprotonated amide nitrogen and some side-chain groups\(^1\). Studies of angiotensin II (ANG II) with Cu\(^{2+}\) and Ni\(^{2+}\) by other analytical techniques have shown that Cu\(^{2+}\) and Ni\(^{2+}\) bind to ANG II at the N-terminal amino group and its three succeeding deprotonated amide nitrogens when the pH of the solutions is above 8.5 and 9, respectively \(^2\)(\(^3\)). Mass spectrometry in general has not been used in investigating interactions between peptides/proteins and transition metal ions. It is the purpose of this paper to show that PDMS is valuable to the study of bindings between ANG II and Pd\(^{2+}\).

ANG II (asp-arg-val-tyr-ile-his-pro-phe) consists of eight amino acid residues and has a molecular weight of 1046 amu. The PDMS mass spectrum is presented in figure 1. The mass region from m/z 240 to m/z 980 is magnified 10 times. In Fig. 1., \((\text{M}+\text{H})^+\) is the base peak, and the most abundant fragment ion corresponds to \((\text{M}+\text{H}-\text{COOH})^+\). Among the peptide sequencing fragments, the \(a_n\) series are detected completely. Other N-terminal ions include several \(c_n\) ions and \(b_n\). Compared to the N-terminal fragments, much fewer C-terminal fragments are detected and these C-terminal fragments are in the higher mass region. The abundances of all the sequencing fragments are less than 10 \% of that of \((\text{M}+\text{H})^+\).

The mass spectrum depicted in Fig. 2 is obtained from an ANG II-K\(_2\)PdCl\(_4\) solution with a 1:1 molar ratio of the peptide and the transition metal ion. The pH of the solution is about 3.5. The features of this spectrum are very distinct from the one of ANG II in Fig. 1. In the molecular ion region, the \((\text{M}+n\text{Pd})^+\) \((n=1-5)\) peaks are displayed in addition to \((\text{M}+\text{H})^+\). The relative intensities of \((\text{M}+n\text{Pd})^+\) to \((\text{M}+\text{H})^+\) decrease dramatically with the increase of \(n\). \((\text{M}+\text{Pd})^+\) is 85 \% and \((\text{M}+2\text{Pd})^+\) is only 11 \% relative to \((\text{M}+\text{H})^+\). In other words, one ANG II molecule can bind to several Pd\(^{2+}\), but only one of the Pd\(^{2+}\) is tightly bound.

In the fragment ion region of Fig. 2, \(a_2+\text{Pd}\) is the dominate ion. Its intensity relative to \((\text{M}+\text{Pd})^+\) is 81 \%. Also, \(a_3+\text{Pd}\), \(a_4+\text{Pd}\), \(a_5+\text{Pd}\), and \(a_7+\text{Pd}\) are detected. Their relative abundances to \((\text{M}+\text{Pd})^+\) vary from 25 \% to 5 \%. The detection of the intense \(a_2+\text{Pd}\) peak proves that a Pd\(^{2+}\) ion is tightly bound to the two amino acid resides at the N-terminal. There are four binding sites available among these two residues: the N-terminal amino group, the carboxyl group in the aspartic acid residue, the guanidinium group in the arginine residue, and the adjacent deprotonated
amide nitrogen. The observation of the abundant \((M+Pd-COOH)^+\) ion suggests that the carboxyl group is not ligated to the Pd\(^{2+}\). The guanidine group in the arginine residue behaves like a glycine residue when the pH is below 10(2). Therefore, we believe that the N-terminal amino group and its adjacent deprotonated amide nitrogen are ligated to the Pd\(^{2+}\) ion. In addition, these two ligand sites are suitable to form a 5-membered ring which complexes to the Pd\(^{2+}\). Due to their high relative abundances, the \(a_n\) series ions provide stronger peptide sequence information compared to the \(a_n\) series shown in Fig. 1. Palladium has several major isotopes which cause the broadened peak appearances. This feature assists in identification of Pd-related fragment series.

![Figure 1. Mass Spectrum of 10\(^{-3}\)M ANG II from PDMS.](image)

![Figure 2. Mass Spectrum of 5\times10\(^{-4}\)M ANG II-K\(_2\)PdCl\(_4\) (1:1 molar ratio).](image)

ANALYSIS OF LARGE PROTEINS BY ELECTROSPRAY MS: APPLICATION TO SOLUBLE COMPLEMENT RECEPTOR

Steven A. Carr, Todd Armbruster, Mark E. Hemling, K. Karl Soneson, and Michael J. Huddleston

This paper details our progress to date on the use of LC-ESMS to confirm the overall sequence fidelity and to establish glycosylation-site usage of a recombinant form of complement receptor type 1 (sCR-1, also known as CD11b) expressed in mammalian cell culture by T-Cell Sciences, Cambridge, Massachusetts. sCR-1 is a 250 kDa glycoprotein that contains thirty tandem repeats of a sixty amino acid domain connected by short (<10) amino acid bridges. The interdomain homology is high, varying from 60 to 69%. There are 25 potential N-linked glycosylation sites. Conventional sequence analysis has been daunting due to the size and complexity of the protein, the very high internal homology, and the presence of an N-terminal block.

The protein was reduced and carboxymethylated (RCM) under standard conditions, and then digested in separate experiments with trypsin alone, and PNGase F and trypsin. Because of the high internal sequence homology, we also explored the use of low frequency cleavage strategies, particularly cyanogen bromide (CNBr) for cleavage at Met. There are 23 Met residues in the protein, and the peptides that would be generated range in molecular mass from ca. 1 to 20 kDa. Our intent was to explore the high mass capabilities of the ESMS system to assign these fragments to unique sequence locations.

The total ion current trace for a typical microbore LC-ESMS analysis of a tryptic digest of reduced and alkylated sCR-1 glycoprotein is shown in Figure 1. The profile is enormously complex reflecting the very large number of peptides and glycopeptides that are generated in a digest of a large protein such as sCR-1, and each chromatographic peak generally contains several components. In order to simplify detection and assignment of peptide components, we have rewritten and coded the Biller-Blemann spectral reconstruction algorithm for use on the Macintosh. This program, which we call "Enhance" resolves LC components that nearly co-elute chromatographically. It does this by determining where in time (i.e., scan number) each mass peak maximizes. The program then eliminates these masses from scans where they have not maximized. This results in dramatically improved apparent chromatographic resolution, and simplified mass spectra. The effect on the above mentioned digest of sCR-1 is illustrated in Figure 1, bottom.

Several procedures have been developed in our laboratory for detection of glycopeptides in the LC-ESMS analyses of glycoproteins. The most recent of these involves LC-ESMS/MS in parent ion scanning mode, and is described elsewhere in this volume (see MP 75, Huddleston et al.). In another procedure, glycopeptides are detected by comparing the LCMS-derived peptide maps of the protein obtained before and after treatment of the glycoprotein with an endoglycosidase, most commonly either PNGase F or Endoglycosidase H. The former is particularly useful as it releases the carbohydrate moiety en bloc from the protein, and converts the attachment-site Asn to an Asp which weighs 1 Da more. The ES mass spectrum of a glycopeptide identified in this manner is shown in Figure 2. Heterogeneity of the carbohydrate moiety of this glycopeptide is readily apparent from the peaks in the (M + 3H)^+ cluster that are separated by the in-chain masses (divided by the charge of +3) of sialic acid and Hex-HexNAc. The spectrum of the former glycosylation-site peptide (not shown) indicates that the attachment site is (either or both) Asn897/Asn447. The mass difference between the former glycosylation-site peptide and the glycopeptide signals indicate a composition of NeuAcα2→3Galβ1→4Galβ1→NAc, dHexα1→HexNAcα1→Hexα1→. Glycopeptides with masses in excess of 6 kDa were directly detected in the LC-ESMS data. Thus far, at least 10 glycopeptides have been identified in this manner.

The carbohydrate moieties of N-linked oligosaccharides in glycoproteins are most often heterogeneous. We have written a program that takes advantage of the resulting predictable peak multiplicity to locate glycopeptides. The program, which we call Peak Pair Search looks for pairs of ions (within a user definable window of scans) separated by user defined mass increments. It is similar to software developed by Andergott et al. for selective location of mass spectra of Si-containing, or halogenated compounds in GCMS. The program can also be used to identify other compound classes such as phosphopeptides which lose, or can be induced to lose, a portion of the molecule indicative of the modification of interest. For glycopeptides we use 146, 162, 203, 291, and 335 (and their corresponding multiply-charged versions, e.g., 281 and 379) corresponding to dHex, Hex, HexNAc, NeuAc, and Hex-HexNAc, respectively. More than 14 glycopeptides have been identified in sCR-1 using this algorithm. An example of the present tabular output is shown in Figure 3. The program can be viewed as a software analog of a constant neutral loss scan.

The CNBr digest of RCM sCR-1 was separated on 2.1 mm C18 reverse phase, and fractions were analyzed either on-line or by infusing aliquots of collected peaks directly into the Sciex electrospray mass spectrometer. Many of the CNBr fractions indicate the presence of complex mixtures of glycopeptides. Although it is possible to assign various molecular weight series to the spectra, correlating the observed molecular weights with specific regions of the sequence has proven more difficult due to the carbohydrate heterogeneity. At present only seven of the 23 possible CNBr peptides ranging in mass from ca. 1000 to 16000 have been assigned.

Data collected in the three LCMS experiments described corroborate ca. 75% of the sequence of this 250 kDa glycoprotein. The LCMS experiments each required only a few hours for data collection rather than several weeks of instrument time as was needed previously for analysis of collected fractions off-line. Data interpretation required approximately one week. This is far faster than we have been able to carry out similar experiments in the past on much smaller proteins.
Figure 1. (top): Total ion current trace of the LC-ESMS analysis of a tryptic digest of sCR-1. Separation was carried out on a 1 mm C-18 column with a TFA/CH₃CN/H₂O gradient at a flow rate of 40 µl/min.
(bottom): The total ion current profile resulting from use of the Enhance algorithm on the above data set.

Figure 2. Electrospray mass spectrum of a glycopeptide identified in the LCMS analysis of a tryptic digest of sCR-1 using comparative LCMS mapping (see text).

Figure 3. Representative output of the program Peak Pair Search for analysis of a small number of scans (525 to 600) from the LCMS data shown in Figure 1. In this case the mass difference analyzed for is 291, the in-chain mass of NeuAc; a scan window of +10 was used. Masses in the delta amu column correspond to the +2 to +5 charge states of 291; delta scan indicates the relative scan number where the second peak (Peak2) of the peak search pair was found.
Covalent attachment of polyethylene glycol (PEG) to enzymes has been shown to prolong plasma half-lives significantly (1) and thereby to improve the therapeutic potential of the enzymes. We have developed and partially implemented a strategy for characterizing PEGated proteins. The strategy involves determination of the number and location of attachment sites as well as the molecular weight distribution of the polyethylene glycol polymers attached. Bovine erythrocyte superoxide dismutase (SOD) was reacted with methoxypolyethylene glycol-succinimidyl succinate with an average molecular weight of 5000 (2) to give the PEG-SOD used in this work. This reaction takes place preferentially with primary amines located on the lysine side chains of SOD linking PEG to lysine via a succinyl group. Treatment of PEG-SOD with base removes the PEG leaving a succinimidyl marker on the lysine. The protease digestion products of reduced and alkylated dePEG-SOD and SOD were subjected to high performance liquid chromatography (HPLC) and tandem mass spectrometry to demonstrate that at least eight of the ten lysines in SOD monomer can be partially derivatized. Figure 1 shows the mass spectra obtained for one of the HPLC peaks from clostripain digestion. The observed m/z 3707.5 corresponds to peptide [78-113] (calc. M$^+$, monoisotopic, 3706.96) which contains one lysine at position 89. The peak at m/z 3789.5 shown in the dePEG-SOD product is 82 higher signaling the presence of the linker. Figure 2 shows the MSMS of m/z 1578.9 found in another of the clostripain digestion of dePEG-SOD HPLC fractions. The fragmentation pattern clearly supports the assignment of peptide [114-126] with a derivatized lysine at position 120. Figure 3 shows electrospray ionization spectra for SOD and dePEG-SOD. The shift from a maximum at +15 for SOD to a maximum at +12 for dePEG-SOD is consistent with lysine derivatization. Analysis of the clusters at each charge suggests that from 2 to 6 PEGs were attached to each SOD monomer. The FABMS and MSMS were made on a JEOL HX110/HX110 high performance mass spectrometer while the electrospray ionization spectra were made with a Vestec source on a Hewlett Packard 5988A quadrupole mass spectrometer.

This work was supported by grants from the National Science Foundation and Sterling Drug Inc.

Figure 1b.

dePEG-SOD/clostridin digest
14.8 min. HPLC fraction

Figure 2.

MSMS of m/z 1578.9

Figure 3.
A polypeptide molecule was constructed from artificially synthesized DNA using recombinant DNA technology. This "artificial protein" was assembled to study the effects that govern crystallization in ordered co-polymers and to test the feasibility of using recombinant DNA technology for the manufacture of totally new polymeric materials. If polymers can be constructed in this way, new materials with protein-like properties can be manufactured.

The polypeptide molecule was constructed using the following sequence of steps:

1) a repetitive sequence of DNA coding for the peptide AGAGAGAGPEG was synthesized;
2) this DNA was introduced into E. coli and amplified;
3) this sequence was isolated from the E. coli and polymerized;
4) the polymers were purified and the 14-mer was spliced between two short DNA sequences coding for polypeptides sequences that could be easily removed by CNBr digestion;
5) the synthetic gene was then inserted into E. coli and expressed; &
6) the protein was purified from the resulting culture.

The sequence -AGAGAGAGPEG- was expected to form one turn of a reverse pleated beta-sheet conformation. Therefore, the polypeptide product was expected to take on a conformation similar to that of naturally occurring fibrous proteins (e.g., collagen).

Definitive analysis of the protein product was problematic. Amino acid analysis was of limited use because of the highly repetitive and segmented nature of the molecule. Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis showed a homogeneous product with an $M_r = 40$ kDa, while the expected $M_r$ of the protein was 17 kDa. NMR and FTIR studies confirmed the presence of the expected side groups but did not give any information about the length of the polymer.

Mass spectrometry was performed at Rockefeller University to determine the structure of the product. The expected product protein contained very few basic residues, therefore electrospray ionization was not feasible. Matrix-assisted laser desorption allowed the characterization of the protein product. The analysis determined that there was an intact product with a molecular mass 56 u higher than expected and that the sample contained a large number of hydrolysis products of the original molecule (~ 50 polypeptides could be identified in the sample). These smaller polypeptides could be removed from the sample by dialysis.

The 56 u discrepancy between the expected and observed molecular species was investigated by analysis of the DNA in the final recombinant E. coli culture. This analysis led to the discovery of two point mutations made by the bacteria, leading to the substitution of two valine residues for two alanine residues. The reasons for this mutation and the identity of the proteolytic agent that led to the formation of the unexpected polypeptide fragments are currently under investigation.
Mass spectrometric analysis of recombinant glycoproteins.


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Structure characterization of biologically active proteins is an integral part of the biotechnology efforts towards the development of novel therapeutic and diagnostic agents by recombinant DNA techniques. Characterization of these molecules with regard to purity and structure presents one of the most difficult and challenging analytical tasks, especially since these proteins are often known to undergo various post-translational modifications, such as glycosylation, phosphorylation and formation of disulfide bonds. The extent and the structure of such post-translational processing depends on the cellular expression system used.

This report describes the application, as well as assesses the analytical utility, of various mass spectrometric techniques (LSI, PD, LD ans ESI) to the structural characterization of the N-linked carbohydrate chains in recombinant human interleukin-4 (IL-4) and interleukin-5 (IL-5) expressed in Chinese hamster ovary (CHO) and NS-1 cells.

In the case of CHO IL-4, LSI mapping of the V8 protease digest of the glycosylated and deglycosylated IL-4 showed that the attachment site of the carbohydrate chain was the first of the two potential glycosylation sites, i.e., Asn-38 (see Proceedings of the 38th ASMS Conference, pp. 1341-2). In these preliminary studies, LSI and PD mass spectrometric analysis of the N-Glycanase-released carbohydrate after derivatization, showed the presence of a fucosylated, disialylated, biantennary complex-type oligosaccharide as the major component. In addition, the PD mass spectrum of the peracetylated carbohydrate exhibited signals corresponding to variations in sialic acid content and/or additional N-acetyllactosamine units due to either higher order branching structures and/or arm extensions as shown in Table I. These assignments agreed well with the oligosaccharide molecular weight information (ca. 2500 Da) deduced by the PD mass spectrometric analysis of the IL-4 glycoprotein prior to and after the enzymatic removal of the carbohydrate moiety. It should be noted that the PD mass spectrum of CHO IL-4 did not provide information on the heterogeneity of the carbohydrate component, even though the presence of heterogeneity was apparent in the doubly and triply charged molecular ions (the singly charged molecular ion was weak).

Analysis of the E. coli and CHO IL-4 by matrix-assisted UV LDMS exhibited an intense M+ signal which was accompanied by weaker signals corresponding to M+2 and 2M+ molecular ions. Even though the size of the carbohydrate moiety was readily determined to be ca. 2 kDa, no definite information on the carbohydrate heterogeneity was available from these data (Figs. 1,2). On the other hand, mass spectrometric analysis of CHO IL-4 by ion spray provided information on the major glycoforms present in the glycoprotein sample. The ion evaporation spectrum showed three envelopes of multiply charged ions (i.e., to 10+) each comprising eight peaks which corresponded to the individual glycoforms of the glycoprotein, as it is clearly shown in the reconstructed spectrum (Fig. 4). For example, the asterisk-denoted ion envelope in Fig. 3 corresponds to the glycoform at m/z 17019 in Fig. 4. This determination is consistent with the mass values derived from the PDMS analysis of the peracetylated oligosaccharide. It should be noted that each glycoform signal in the ion evaporation spectrum was accompanied by an adduct ion 98 Da higher, possibly due to the addition of H2SO4. Therefore, the MW determination of the glycoprotein by the ion-spray ionization method, provided more information on the existing glycoform pattern. Nevertheless, in the analysis of the larger dimer- form of the IL-5 glycoprotein derived from CHO and NS-1 cell lines, no useful ion-spray data were obtained. On the contrary, UV LDMS analysis of the CHO IL-5 gave an intense signal at ~30700 Da with extensive heterogeneity. This mass value compared with the calculated value derived from the cDNA-derived protein sequence, gives an approximate value of 2200 Da for the size of the N-linked carbohydrate component per monomer. Similarly, the observed LD mass value for the NS-1 derived IL-5 was ~31500 Da, thus indicating that the size of the attached carbohydrate component is ~800 Da higher than that in CHO IL-5, possibly due to an additional O-glycosylation.

These results demonstrate the synergistic role of the ion spray and high-energy desorption ionization techniques, which in combination with enzymatic or chemical cleavage of the carbohydrate side chains can provide the composition of these side chains.
**Oligosaccharides Corresponding to Observed PD-MS Molecular Weights (MNe+)**

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**Figure 1**

UV-LASER DESORPTION MASS SPECTRUM
Interleukin-4
(M.W. 14956)

**Figure 2**

UV-LDMS
CHO IL-4

**Figure 3**

ION SPRAY MASS SPECTRUM
CHO IL-4

**Figure 4**

Reconstructed Spectrum
CHO INTERLEUKIN-4

Authors: Wei-Lung Shen and R. D. Satzger; U.S. Food and Drug Administration, National Forensic Chemistry Center (NFCC), 1141 Central Parkway, Cincinnati, OH 45202.

Atmospheric pressure plasma ion sources, such as direct current plasmas (DCP), inductively coupled plasmas (ICP) and microwave induced plasmas (MIP), have been used successfully for elemental mass spectrometry. However, only a few papers have dealt with the use of plasmas, such as the ICP(1) or the MIP(2-4) as sources for generating molecular ions. The original research using an atmospheric pressure ICP(1) and low pressure MIP(2) as molecular ionization sources produced extensive fragmentation. Potential advantages of using the MIP rather than the ICP as a soft molecular ion source include a relatively low plasma temperature and the flexibility to operate with various plasma support gases (such as He, Ar, N$_2$) and mixed gas (such as He/N$_2$, Ar/N$_2$, etc.). Generation of molecular ions using an atmospheric pressure plasma is difficult because the high excitation and ionization energy of the plasma produces considerable fragmentation. Recently, two reduced pressure MIP systems(3,4) operating at 1 to 2 torr did provide a means of using a plasma as a source of molecular ions. However, sample introduction into a low pressure MIP is limited to gaseous samples.

Advantages of an atmospheric pressure ion source include its simple design and ease of sample introduction because it can be easily interfaced directly to real world systems without a vacuum barrier. Since the majority of effluent from any chromatographic experiment consists of solvent, most of this can be eliminated to the atmosphere and not introduced to the vacuum chamber, resulting in reduced sample memory or contamination of the vacuum system. The API source utilizes conventional sample introduction techniques, and plasmas operating at atmospheric pressure can easily be sustained during introduction of a variety of sample matrices.

In this report, a new plasma API source for mass spectrometry, which generates molecular ions at atmospheric pressure, has been developed using a low power atmospheric pressure helium microwave-induced plasma (Figure 1). This new plasma API source design was developed from the Microwave Induced Plasma - Tantalum Injector Probe (MIP-TIP) which is described in a previous report(5). The plasma API source sustains a helium plasma at low (10-30 W) power levels and produces quasi-molecular parent ions and molecular fragments of analyte at atmospheric pressure. The design of this source enables soft molecular fragmentation with either gaseous (Figure 2, 3 and 4) or aqueous (Figure 5) sample introduction at atmospheric pressure. The potential for interfacing this plasma API source with liquid and gaseous chromatographic techniques will be discussed.

References
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

**Figure 1** Low power microwave induced plasma API source.

**Figure 2** Gaseous PFTBA sample introduction.

**Figure 3** Gaseous PFTBA sample introduction.

**Figure 4** Gaseous PFTBA sample introduction.

**Figure 5** Liquid sample introduction - Caffeine.
A comparative study of photo-ionization and electron impact ionization on hydrocarbons is of importance to evaluate the potential of photo-ionization mass spectrometry in the analysis of complex mixtures of hydrocarbons. The fixed energy of photons guarantees a high degree of reproducibility in the ionization and fragmentation processes. The energy of the photons can be set just above the ionization potential of the hydrocarbons, generating almost only molecular ions. However, the small number of photons available, roughly $10^{12}$-$10^{13}$ per second, and the relatively small cross sections for photo-ionization result in low ion currents. These low ion currents restrict the sensitivity of the analytical method. A drawback which can be partially overcome by using ion counting techniques.

Low energy electron impact ionization as an alternative for inducing limited fragmentation has various practical difficulties when using analytical mass spectrometers. Ionization by a well-defined low electron energy is difficult to achieve because of the lack of control on the energy spreading of the electrons. This energy spreading is mainly due to the potential across the filament. This problem impairs the ionization energy selectivity of the electron impact technique.

The instrument used in the experiments has already been described fully [1]. To test the performance of the photo-ionization source for the analysis of hydrocarbons, the total ion currents for electron impact ionization and photo-ionization for several linear alkanes (n-pentane up to n-decane) are measured. Also the pressure inside the ion source and the temperature of the ion source are measured. Taking two accurate measurements for the absolute total photo-ionization and electron impact ionization cross sections from literature, the total transmission loss of the instrument is determined. For the photo-ionization source one out of every 1500 ions created in the source is measured by the detector. For the electron impact ionization source this ratio is better. Out of every 180 ions created in the ion source, one ion is detected.

Having calculated the total transmission loss, we determined the absolute total photo-ionization and electron impact ionization cross sections of n-pentane up to n-decane. These are shown in figure 1 as function of the molecular weight. The absolute values and the gradual rise of the cross sections as function of the molecular weight are in agreement with literature [2,3].

A semi-empirical model is developed describing the total electron impact ionization cross section as function of the electron energy of alkanes, alkenes, alkynes and cyclo-alkanes. This model is based on the 'additivity rule', but uses two extra terms. One term to describe the CC- and CH-coupling and a second term to account for the effect of the double and/or triple bonds. In figure 2, the model is compared to several measured total electron impact ionization cross sections of alkanes as function of the electron energy. The model is represented by the solid line. The experimental values are represented by symbols referring to different measurements. We observe as function of energy a good fit of the model to the measured cross sections. The model also describes within the experimental uncertainty (15%-20%) the behaviour of the total electron impact ionization cross sections of alkenes, alkynes and cyclo-alkanes as function of the electron energy.

Methods to produce coherent vacuum ultraviolet radiation have been available for almost two decades. Widespread application to mass spectrometry, however, has occurred only recently. The method used in our laboratory to generate tunable radiation by third harmonic generation is shown in Figure 1. Tunable radiation in the 611 to 532 nm region is generated by a Nd:YAG laser pumped dye laser system. The visible radiation is frequency mixed with residual 1064 nm radiation from the Nd:YAG laser to produce radiation in the 355 to 388 nm region. The ultraviolet radiation is focused in a cell containing one or more rare gases to generate third harmonic radiation in the 129 to 118 nm (9.6 to 10.5 eV) region. The cell length of one meter is based upon a design developed by C.H. Becker and coworkers at SRI International. Vacuum ultraviolet radiation is generated collinearly with the ultraviolet radiation. Separation of the two is accomplished by sending them through the second lens off-axis. The difference in refractive index between the two wavelengths causes the two beams to be physically separated in the source region. Since the ultraviolet radiation passes through the source region away from the axis defined by the acceleration grids, ions produced by it are not detected.

![Figure 1](image-url)
The third harmonic power, $P_{3\omega}$, is given by the equation

$$P_{3\omega} \propto |X(3)|^2 N^2 P_{\omega} F$$

where $X(3)$ is the third order susceptibility, $N$ is the number density of the nonlinear medium, $P_{\omega}$ is the pump laser power, and $F$ is a phase matching function. Since $X(3)$ and $N$ are small, the conversion efficiency is usually low. $F$ is nonzero only when the medium exhibits negative dispersion. Therefore, continuous tuning across the vacuum ultraviolet region is not possible by this method. In our work, xenon is used to generate radiation at 118.2 nm and in the 127 to 129 nm region, while krypton is used to generate radiation in the 121 to 123 nm region. The conversion efficiency is optimized by mixing appropriate amounts of xenon (or krypton) with argon in the cell to maximize the product $N^2 F$ in the above equation.

Coherent vacuum ultraviolet radiation has several useful characteristics for photoionization including high power ($10^{11}$ to $10^{12}$ photons/pulse), narrow bandwidth ($\leq 1 \text{ cm}^{-1}$), low divergence (allows the radiation to be confined to a small spatial region), wavelength tunability (other four-wave mixing processes can provide continuous tuning throughout the vacuum ultraviolet region), and short pulse width ($\leq 6 \text{ ns}$; can be synchronized with other pulsed beams).

In our laboratory, this radiation is used for several experiments including photodissociation-photoionization mass spectrometry, photoionization of laser desorbed neutrals, and studying the rate of ion decomposition vs. molecular size. In photodissociation-photoionization mass spectrometry, molecules are dissociated with an excimer laser beam and the neutral products are softly ionized with coherent vacuum ultraviolet radiation. This method can be used to study neutral photochemistry and to distinguish isomeric structures. Photoionization of laser desorbed neutrals allows the mechanism of laser desorption to be studied. Kinetic energy distributions of neutrals desorbed from the surface can be determined from the delay time between the desorption and photoionization lasers. Internal energies of desorbed neutrals can be determined from the wavelength dependence of fragmentation in the photoionization mass spectrum. Elucidating the molecular size dependence of primary fragmentation is simplified with single photon ionization since $P(E)$ for ionization can be independent of molecular size and the photon energy can be adjusted to eliminate secondary fragmentation. Under these conditions, changes in the number of metastable product ions with increasing molecular size give an indication of the parent ion unimolecular decay rate dependence upon molecular size. Each of these areas is discussed in more detail in other conference presentations.
PULSED FAST ATOM BOMBARDMENT SAMPLE DESORPTION WITH MULTIPHOTON IONIZATION IN A SUPERSONIC JET/REFLECTRON TIME-OF-FLIGHT MASS SPECTROMETER

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Multiphoton ionization mass spectrometry (MPIMS) has been shown to be a very powerful technique for chemical analysis. Advantages of using MPI as an ionization source for mass spectrometry include high sensitivity, high selectivity and the ability of controlling mass fragmentation patterns. In this paper, an instrument for performing MPIMS and supersonic jet spectroscopy (SJS) studies of biological molecules and high molecular weight polyaromatic hydrocarbons (PAHs) is described. Specifically, pulsed fast atom bombardment (FAB) has been developed as a means of vaporizing thermally labile biological molecules and PAHs into the gas phase. The resulting neutral molecules are entrained into a CO₂ supersonic jet which prevents thermal decomposition by collisional cooling and carries the molecules into a time-of-flight mass spectrometer (TOFMS) where resonant two-photon ionization (R2PI) is performed by a UV laser beam.

Figure 1 shows the schematic diagram of the experimental setup for FAB/SJ-MPI. The system consists of an angular reflectron TOFMS (R.M.Jordan Co.) mounted vertically in a six-port cross pumped by a 6-in. diffusion pump. A pulsed nozzle with a 50-μs pulse width is used to form a supersonic jet. CO₂ is used as the expansion gas throughout this work. The jet expands into the acceleration region of the TOF and a laser beam perpendicular to both the jet and flight tube ionizes the sample. No skimmer was used in this study. The 1-meter-long flight tube is differentially pumped by a 4-in. diffusion pump. The pressure in the flight tube is below 2x10⁻⁷ Torr and the pressure in the ionization region is <10⁻⁶ Torr. The ionization source is a frequency quadrupled Nd:YAG laser (GCR-3, Spectra-Physics, CA) which generates a 266-nm radiation with a 7-ns pulse width. The laser is operated at 3 Hz repetition rate. A cylindrical lens is used to focus the laser beam to the mass spectrometer. The mass spectrum is recorded by a LeCroy 9400A digital oscilloscope, and data are stored in an IBM compatible 386SX PC for processing. Figure 1.

FAB/SJ-MPI is performed with a saddle-field FAB gun which is fitted into the test chamber through a 2-in. flange and placed close to the nozzle orifice. The distance between the FAB gun outlet and the nozzle orifice is about 40 mm. The distance between the FAB gun and the center axis of the jet is also about 40 mm. A 5-mm or 10-mm diameter sample probe made of machinable Macor ceramic is located at about 20 mm away from the center axis of the jet. About 100 μg sample is generally used and placed on the sample probe directly by either wetting or dissolving the compound in a solvent (i.e. methanol) and coating on the surface with the use of a spatula. Argon is used in the saddle-field discharge for the FAB gun. The anode potential is fixed at 10 kV and, by adjusting the pressure in the gun, the discharge current varies from 1 to 5 mA. The size of the FAB beam is approximately 1.5 mm in diameter. The high voltage power supply for the FAB gun is Spellman Model RHSR15PNS0 which is operated in a pulsed mode using an external trigger system. The actual sequence of events is controlled by several delay generators. The FAB gun is first turned on to desorb the sample, the pulsed nozzle opens to form a supersonic jet, and then the sample is entrained into the jet and carried into the ionization region of the TOFMS where the ionization proceeds.
In this presentation, we have shown that a variety of biological molecules can be studied without significant thermal decomposition, with both soft and hard ionization mass spectra obtainable. The chemicals studied by this FAB/SJ-MPI technique include amino acids, carboxylic acids, catecholamines, neuroleptic drugs, pineal indoles, small peptides, PAHs, and organic salts. For dipeptides, it is found that although the molecular ion peak can be observed, the ion peak at 18 mass units lower sometimes is the dominant peak in the mass spectrum. This interesting result is also obtained with laser desorption/SJ-MPI. However, one significant advantage of this FAB method over laser desorption is that the FAB gun can be easily constructed and maintained at a relatively low cost. In addition, we show that high molecular weight PAHs can be readily desorbed by FAB and studied by R2PI. The detection limit in our present system is found to be in the low nanogram regime. We have also demonstrated that this technique can be used for selective detection of the active substance in a drug tablet. Finally, we have shown that this technique provides a unique means for the studies of chemical reactions induced during the FAB desorption process. Figure 2 shows the MPI mass spectrum of dimethyl-4-phenyl piperazinium iodide. The direct FAB ionization mass spectrum of the same compound is shown in Figure 3. The FAB mass spectrum in Fig.3 displays a base peak at m/z 191, which is the molecular cation from the organic salt minus the iodide. However, the MPI mass spectrum shows the base peak at m/z 176. This peak is from the neutral species generated during the FAB desorption process. It is the organic salt minus CH4. The hard ionization mass spectrum shown in Fig. 2 is very useful for the structural analysis of the organic salt. With the combination of FAB and FAB/SJ-MPI, we can now detect not only the ions but also the neutrals and/or radicals produced during the FAB process, which is useful for the mechanistic studies of FAB.
The impact of key polaronic properties on collision studies through an indirect approach using the collision of secondary ions. The collision of secondary ions with atomic targets is an efficient method for investigating short-range atomic properties.

In our study, we use a novel approach to examine the collision of secondary ions from an energetic beam. The secondary ions are accelerated to energies that are in the range of several hundred eV. The ions are then allowed to collide with an atomic target. The collision results are analyzed using mass spectrometry.

The main finding of our study is that the polaronic properties of the secondary ions have a significant impact on the collision outcomes. This is particularly true for ions that are highly charged.

The results of our study suggest that future research should focus on developing new approaches to study polaronic properties using collisions. This could include the use of different target materials or the use of different ion beams.
enhancement is calculated as:
\[ e = \frac{Y_n(M)}{(2n+1)Y_0(M)} \]
where \( Y_n(M) \) is the yield of M from the \( n^{th} \) cluster (either Cs(CsI)\( n^+ \) or (CsI)\( n^+ \)) and \( Y_0(M) \) is the yield of M from the \( 0^{th} \) cluster. The enhancement in the yield of I" and Cs\( ^+ \) from cluster impacts was calculated at a projectile velocity of 0.04 keV/amu for the five projectiles available. The enhancement was found to be exponentially dependent on the number of atoms in the projectile over the range studied (1 to 9 constituent atoms). Though one might expect that the enhancement would plateau at a large number of constituent atoms, this was not observed in the range studied. Enhancements as large as -20 (with \( n = 4 \)) were observed. This corresponds to an increase in the yield of two orders of magnitude over that from the atomic projectile.

In addition to the yield enhancement, coincidence counting can be used to observe correlations between secondary ions. Once a secondary ion spectrum is obtained, species emitted coincidentally with an ion of interest can be identified and recorded in a coincidence spectrum. Comparing this coincidence spectrum with the secondary ion spectrum, it is possible to determine the relationships between the secondary ion of interest other ions in the spectrum. To quantitate these relationships, a correlation coefficient, \( Q \), is calculated:

\[ Q = \frac{N(A,B)S}{N(A)N(B)} \]
where A is the ion of interest, \( N(A,B) \) is the number of counts in peak B in the coincidence spectrum, \( S \) is the number of primary ions, and \( N(A) \) and \( N(B) \) are the number of counts in peaks A and B in the secondary ion spectrum. A correlation coefficient of one indicates independence of the ions from one another. A correlation coefficient greater or less than one indicates a correlation or anticorrelation between the ions. We chose to examine correlations between I" and other secondary ions desorbed by atomic and cluster projectiles. It was found that whether the projectiles are polyatomic or atomic, I" and hydrocarbon ions are independent (\( Q = 1.5 \)) whereas I" and (CsI)\( n^+ \) cluster secondary ions are strongly correlated (\( Q = 3 \)). This correlation indicates a chemical relationship between the I" and (CsI)\( n^+ \) ions and should be an indicator of the mechanism of ion formation. One possible mechanism would produce the ions by metastable decay. In this case, a large, multiply charged, cluster ion would be desorbed and rapidly decay to form I" and smaller cluster ions. These ions would then be correlated with one another.

This study demonstrates the usefulness of coincidence counting TOF-MS for obtaining additional information about the sample, secondary ions, and perhaps the ion formation process. The potential usefulness of clusters as projectiles is also apparent. These projectiles may be particularly well suited for molecular mapping of surfaces. Cluster ions produce higher yields, thus fewer primary ions are needed to obtain a given signal.

It has been shown a few years ago [1,2] that molecular ions can be desorbed from a plane surface when a constant electric field is applied between the sample and a grid placed at a small distance from this sample. No external ion source is needed to measure a time of flight mass spectrum between electrons and ions emitted simultaneously from the surface. A very simple arrangement can be used for analytical measurements [3] or for generating primary ions in SIMS [4]. The name "Spontaneous Desorption Mass Spectrometry" (SDMS) was given originally by us because of the unknown origin of the observed phenomena. Experimental investigations of the mechanisms have been pursued later on [5,6].

In ref. [2], evidences for secondary ion (SI) emission mechanisms were observed. Primary projectiles would originate from the grid and strike the sample with an energy equal to the potential difference applied between the sample and the grid. Field emission processes are involved in SDMS. Above a certain field value, SD occurs and the molecular ion yield is defined as the ratio between the number of molecular ions detected in a TOF spectrum and the number of electrons. The molecular secondary yield in SD is larger than the yield measured for atomic impacts on the same solid sample target (with Cs ions for example). This has suggested that cluster projectiles could hit the sample since it is known that large SI yields are observed under cluster impacts [8].
An experimental arrangement has been set up where both type of measurements: SD mass spectrometry and cluster induced desorption mass spectrometry, can be performed - alternatively - in the same experimental conditions (same sample, vacuum, detectors,...). Different organic molecules have been used as projectiles (from mass 50 to 166) at an energy equal to the accelerating potential in SDMS. It is concluded that molecular ions with masses around 60-70 could be the main primary projectiles emitted from the grid in SDMS. These ions originate from an organic contamination of the grid. Secondary ions in the same mass range have been observed when bombarding the grid by fission fragments.

Fig. 1a shows a normal SD TOF spectrum of a phenylalanine sample.

The time measurement is triggered by electrons produced by molecular ion impacts on the phenylalanine target. A time window ($\Delta t = 50$ nsec, $W_1$ in Fig. 1a) has been defined a few hundreds of nsec after the first triggering electrons and this time window has been used to define a new origin of time for a new time of flight spectrum where all time correlated events are being recorded during data acquisition. Fig. 1b shows this TOF spectrum that is similar to the total TOF spectrum in Fig. 1a. This means that shortly after a first impact on the target, another impact may occur that induces another simultaneous emission of electrons and ions. Several time windows can be set at different time values after the first triggering event. The yield of emission corresponding to these delayed impacts has been plotted in Fig. 2 as a function of time.

The bombardment of the same sample by well known molecular ions did not exhibit delayed emission effects. Therefore, in SDMS, the delayed emission of secondary particles - that gives rise to a smaller yield - may be due to:

1) in-flight fragmentations of small molecules during their acceleration from the grid. The neutral fragments arrive later at the surface with a smaller energy.
2) delayed emission from the grid after a very small micro-discharge.

Development of a Compact Tandem Time-of-Flight Mass Spectrometer for the Analysis of Small Biomolecules

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A compact, versatile TOF-TOF mass spectrometer is currently under development for the purpose of studying small biological compounds. The dual reflectron instrument will incorporate a collision cell providing MS/MS capabilities. A key component of this design is the ability to independently adjust the flight tube potentials so that high, low, or reactive collisions may be utilized. The source is designed so that a number of different probe types and extraction optics can be employed in order to test a variety of laser desorption conditions and geometries.

Divided into two stages, the first part of this study (discussed here) involves testing of the linear and single reflectron geometries using an assortment of probe designs and laser conditions to optimize resolution and sensitivity. The second will incorporate the double reflectron geometry with the collision cell to allow MS/MS capabilities. Additionally, time delayed extraction will be employed to allow metastable decomposition to occur prior to initial acceleration.

The test probes include a 90° off-axis desorption source, a parallel prism, and a parallel grazing incidence design. The ion optics for the off-axis probe consist of a two-stage draw out region as prescribed by Wiley-McLaren space focussing conditions. While very high voltage extraction is desirable to minimize effects of desorption kinetic energy spread in the TOF peak widths, the asymmetry of this design in the source region (and therefore asymmetric field lines) requires the use of low voltage extraction in order to acquire precisely reproducible signals. Results indicate that under these conditions, the resolution is limited by the energy spread induced by laser desorption and would not be suitable for high resolution applications. The parallel prism probe is based on a design by Reilly et al. (see Analytical Instrumentation, 16, 133 (1987)) in which the sample is applied to the hypotenuse face of a right angle prism. This surface is coated with a thin layer of aerosol graphite to define the source potential. A laser beam is directed to its interior side at grazing incidence, ionizing the sample with no above-surface interference. Theory indicates that this technique can produce extraordinary TOF resolution if very short laser pulses are employed, although initial studies here indicate that the TOF peaks of samples applied to the surface are significantly broader than components found in the thin graphite layer (salts and propellant). The third probe is a flat metal surface oriented opposite to the ion extraction lenses with the ionizing laser directed at grazing incidence to this surface. Ions are extracted at high voltage (~2 kV) followed by deceleration to their drift velocities producing TOF peaks which appear to be limited by the laser pulse duration. This third design is likely to be incorporated for future studies.
Laser

Tandem Time of Flight MS
Surface-Induced Dissociation in a Hybrid Mass Spectrometer

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Aberth has recently reported the use of a microchannel plate to achieve surface-induced dissociations (SID) in a Wien tandem mass spectrometer(1). Compared to gas collision processes, surface-induced collisions convert a larger amount of the incident ions translational energy into internal energy(2) and reduce the background pressure in the mass spectrometer. In the case of hybrid mass spectrometers there may be increased fragment ion transmission, particularly at low mass, if the fragment ions are formed at positions close to the axis of the Rf-only quadrupole(3). This can be achieved if the parent ion beam is focused onto the micro-channel plate at the entrance of the Rf-only quadrupole.

A Kratos CONCEPT SQ hybrid mass spectrometer was readily modified for microchannel plate SID studies by attaching commercially available microchannel plates to the entrance lens aperture of the Rf-only quadrupole.

Figure 1 shows a unimolecular decomposition spectrum (no collision gas) for triazine (m/z 1466). Figure 2 shows a daughter ion spectrum for triazine at 150eV with collision gas present and Figure 3 shows the spectrum for microchannel plate SID. It can be seen on comparison of Figures 2 and 3 that there is a significant increase in relative intensity of low mass fragments in the SID spectrum. This is probably due to the higher amount of energy available for conversion into internal energy in the collision, but may also have a contribution from better transmission at lower mass.

Transmission through the microchannel plate was, however, very low (<1%). Comparable quality SID spectra were obtained using wire meshes giving transmission of the order of 40%.

![Figure 1: Unimolecular (no collision gas) daughter ion spectrum of triazine](image-url)
Figure 2: Daughter ion spectrum of triazine from EBQQ

Figure 3: Daughter ion spectrum of triazine from EB(SID)QQ

References

Low Energy Ion/Surface Reactive Collisions Investigated in a BEEQ Hybrid Mass Spectrometer

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Murray Hill, NJ 07974

A recently completed BEEQ hybrid mass spectrometer has been utilized to investigate ion/surface reactive collisions as a function of the nature of the surface and of the primary projectile's collision energy. Figure 1 is a diagram depicting the important ion optical elements of the instrument. With this arrangement, the primary ion collision energy can be varied from < 5 eV to 3000 eV. The low energy ion/surface reactive collisions presented were performed in the collision energy range of 2 eV to 100 eV. Experiments were performed using a number of specially prepared target surfaces ranging from nascent stainless steel to surfaces in which C-16 protonated or perdeuterated alkyl thiols are covalently bound to a gold substrate. The preparation of these surfaces results in the formation of self assembled monolayers (SAM) in which the bound molecules are oriented vertically. A surface consisting of CF3(CF2)11(CH2)2SH covalently bound to Au has also been investigated.

Experiments in which the pyrene molecular ion impinges upon a nascent stainless steel surface at various collision energies (30-100 eV) indicate the formation of C6 and C8 adducts. Figures 2a and 2b are representative reactive collision spectra for the pyrene molecular ion (m/z 202) at a) 50 eV and b) 100 eV impacting collision energy. The nature of these adducts appear to be dependent upon the impacting energy, indicated by the formation of the (M+11)+ and (M+13)+ ions at a collision energy of 50 eV, while the (M+9)+ and (M+11)+ ions are more abundant at a collision energy of 100 eV. Experiments in which the molecular ions from benzene and other polycyclic aromatic hydrocarbons (PAH's), such as naphthalene and anthracene, impinge upon a stainless steel surface also show formation of (M+CH3-nH2)n+ reaction product ions, where n is the number of H2 molecules lost. This trend is seen quite frequently for the production of the fragment ions from various PAH's, i.e., a stable CnH8+- and CnH7+ series as well as a CnH2+ and CnH3+ series are seen in the CID, SID and LD spectra of these molecules.

The above experiments were performed off of a stainless steel surface which most certainly contained various adsorbates on the uppermost portion of the surface. In order to perform more controlled experiments, i.e., the nature of the surface being well characterized, the molecular ion of pyrazine was used to investigate ion-surface reactive collisions with the SAM surfaces. Using the monolayer surface consisting of the C-16 deuterated thiol, the most abundant reactive collision products observed were (M+D)+ and (M+CD3)+, while the C-16 protonated surface yields (M+H)+ and (M+CH3)+. Figures 3a and 3b are representative reactive collision spectra for the pyrazine molecular ion impinging upon a) a C-16 perdeuterated thiol SAM and b) a CF3(CF2)11(CH2)2SH SAM. As noted, the interaction of the pyrazine molecular ion with the C-16 perdeuterated surface yields abundant reaction ions. However, the fluorinated monolayer surface suppresses the reaction channel and, in fact, enhances the fragmentation channel. Results from these experiments suggest a possible mechanism which may proceed via electron transfer to the incoming ion to yield an adsorbed ionized alkane. Further studies are underway to test this hypothesis.

References

Figure 1

Figure 2a

Figure 2b

Figure 3a

Figure 3b
When analyzing complex samples using GC/MS it is very common to encounter components which are closely eluted. Most GC/MS data systems incorporate a Biller-Biemann type algorithm to help resolve spectral components in these situations. Successful application of this technology however, is limited to components with retention time differences greater than two scans. An alternative technique described by Rindfleisch allows the separation of components with retention time differences of as little as 1.5 scans but the computational overhead has limited its application. The Rindfleisch algorithm is capable of separating spectra of adjacent components which are closer because it involves estimating the retention time for each mass chromatogram maximum based on its peak shape rather than identifying the scan with the most intensity.

Using the Rindfleisch algorithm as a conceptual starting point, computational experiments were undertaken to determine if more precise methods for calculating mass chromatogram retention times could be identified and implemented in one of today's GC/MS data systems. This was done using a data set acquired on a VG Trio-I scanning from 35 to 500 amu once per second and using a 30 meter DB-5 column. Decafluorotriphenylphosphine was selected as the test compound because it provides a large number of mass peaks throughout the acquisition mass range and thus represents a worst case situation.

It was determined that acceptably reproducible mass chromatogram retention time centroids could be calculated by fitting the three intensities centered at the most intense value of a maximum to a second order curve. Using all mass peaks with intensities greater than or equal to one percent of the base peak, 68 peaks in all, the calculated centroids had a standard deviation of 0.04 scans (seconds). Based on this precision value, it is anticipated that two components with retention times which differ by 0.12 scans (seconds) should have spectra which are separable with 99 percent confidence. Testing of this algorithm on experimental data has shown that spectra for components eluted with centroid groupings as close as 0.48 scans can be resolved (Figure 1) and that peaks containing as many as six components in the space of 8.4 scans (Figure 2).

Acknowledgements: The author would like to thank VG MassLab Ltd. for coding the described algorithm into an experimental software set for testing.

References:
Figure 1. Spectral separation of hexadecane-$d_{14}$ from hexadecane-$d_{13}$, which have a mean mass chromatogram centroid difference of 0.48 scans (seconds).

Figure 2. Spectral separation of six overlapping components in a window 8.4 scans seconds) wide. Separated spectra for the three closest components, biphenyl, n-tetradecane and 2-chloronaphthalene, are shown.
Comparison of Calibration Methods for Multiple Tracer Studies

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Rates for substrate kinetics from constant infusion of a tracer are determined by measuring the mole ratio of labeled tracer to unlabeled tracee after infusion of the tracer. Calibration of mass spectrometer responses to the mole ratio of tracer to tracee using linear regression is commonly used with one tracer and gives good results.

With multiple tracers the calibration is more problematic. Tracers which differ from one another and/or the tracee by only one mass unit often contribute significant amounts of ion abundance into the signals for the other isotopomers. Loss of label during derivatization, or during synthesis results in less than the theoretically derived abundances for the ions of the analytes. Finding useful combinations of tracers where the abundance of the mass measured does not overlap with one of the other tracers, is sometimes not possible or at best, expensive. In response to this problem, we have investigated a number of methods for accurately calculating mole ratios in multiple tracer studies with tyrosine, leucine and lysine.

In our studies of phenylalanine substrate kinetics, subjects were infused with both 13C phenylalanine and d2 tyrosine. The amino acids were isolated from plasma and derivatized as the heptafluorobutyryl propyl derivative using literature methods. A set of 24 calibration samples was prepared covering the expected range of tracer:tracee mole ratios for 13C phenylalanine, 13C tyrosine and d2 tyrosine. The calibration and plasma samples were analysed by three replicate injections into a HP 5988 GC-MS (methane NCI). The data was divided into two groups; the first and last injections were combined into a large calibration set. The predicted mole ratios from the second injection were calculated using calibration functions obtained by various methods. A small subset of this data was used as a mock daily calibration set. The mean squared error was used to compare the predictive accuracy of various methods including: overlap correction (1) which utilises an arithmetic correction for overlap, K matrix method (2) which is the classical linear regression method, Brauman's least squares (3), and the P matrix method or forward calibration (4). The results for these methods using various numbers of predictor variables for d2 tyrosine are:

<table>
<thead>
<tr>
<th>Method</th>
<th>MSEP</th>
<th>MSEP *</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>P matrix, 1 predictor</td>
<td>0.3018</td>
<td>0.6000</td>
<td>0.9687</td>
</tr>
<tr>
<td>2 predictors</td>
<td>0.0308</td>
<td>0.1754</td>
<td>0.9975</td>
</tr>
<tr>
<td>3 predictors</td>
<td>0.0279</td>
<td>0.1670</td>
<td>0.9976</td>
</tr>
<tr>
<td>daily</td>
<td>0.1027</td>
<td>0.3204</td>
<td></td>
</tr>
<tr>
<td>Overlap correction</td>
<td>0.1199</td>
<td>0.3464</td>
<td>(0.9943)</td>
</tr>
<tr>
<td>K matrix, 2 predictors</td>
<td>0.0918</td>
<td>0.3031</td>
<td></td>
</tr>
</tbody>
</table>

and for 13C Tyrosine:

<table>
<thead>
<tr>
<th>Method</th>
<th>MSEP</th>
<th>MSEP *</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>K matrix, 1 predictor</td>
<td>0.1052</td>
<td>0.3243</td>
<td>0.9943</td>
</tr>
<tr>
<td>2 predictors</td>
<td>0.0958</td>
<td>0.3096</td>
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<tr>
<td>P matrix, 2 predictors</td>
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<td>0.1754</td>
<td>0.9953</td>
</tr>
<tr>
<td>daily, 2 pred</td>
<td>0.2421</td>
<td>0.4921</td>
<td></td>
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<tr>
<td>Brauman's Least Squares</td>
<td>0.1098</td>
<td>0.3313</td>
<td></td>
</tr>
</tbody>
</table>

The improvement using multiple predictors (abundance ratios) is marked. The lack of accuracy with one predictor is expected because of ion overlap.
1,3C, 1,2,3C, and 5,5,5-d3 leucine (L1, L2, L3) in a 4X4 calibration matrix (.01-2.5 mole percent) were measured by GC-MS-EI as the N-trifluoroacetyl methyl ester at m/z 183-185, 140-144, and 154-156. Principal Components regression (4) extracted the calibration function from the abundances even though one or more tracers were contributing abundances to the signal. Results for this calibration are:

<table>
<thead>
<tr>
<th></th>
<th>R^2</th>
<th>R^2 pred</th>
<th>R^2 validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>0.9913</td>
<td>0.9814</td>
<td>0.9805</td>
</tr>
<tr>
<td>L2</td>
<td>0.9976</td>
<td>0.9952</td>
<td>0.9917</td>
</tr>
<tr>
<td>L3</td>
<td>0.9984</td>
<td>0.9961</td>
<td>0.9925</td>
</tr>
</tbody>
</table>

A similar approach was used for a four tracer lysine study. Four sets of ions were used to model the calibration space modeled by the 5X5 calibration matrix. Partial least squares regression (PLS2) of the data gave the following results:

<table>
<thead>
<tr>
<th></th>
<th>R^2</th>
<th>R^2 pred</th>
<th>R^2 validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>0.9901</td>
<td>0.9805</td>
<td>0.9782</td>
</tr>
<tr>
<td>L2</td>
<td>0.9925</td>
<td>0.9912</td>
<td>0.9897</td>
</tr>
<tr>
<td>L3</td>
<td>0.9941</td>
<td>0.9926</td>
<td>0.9915</td>
</tr>
<tr>
<td>L4</td>
<td>0.9956</td>
<td>0.9939</td>
<td>0.9879</td>
</tr>
</tbody>
</table>

HETEROLYTIC ENERGIES OF X-ALKYL BONDS

Yu-Ran Luo* and Philip D. Pacey, Department of Chemistry,
Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J3

Following an approach taken earlier in estimating the homolytic BDEs of X-alkyl bonds, we have found that there are good linear relations between the differences \( \Delta H^o(X^-C^+(CH_3)_mH_{3-m}) \) and \( \Delta H^o(X^-CH_3^+) \) and the covalent potential of X, \( \psi_x \), which is a new scale of electronegativity by Luo and Benson.\(^1,2\) Here X is Cl, Br, I, H, OH, SH, NHg and CHg and m is the degree of methyl substitution in the group C(CH\(_3\))\(_m\)H\(_{3-m}\), which is ethyl, iso-propyl or tert-butyl when m = 1, 2 or 3, respectively. Then the heterolytic BDEs of X-C bonds can be reproduced by the following equation:

\[
\Delta H^o(X^-C^+(CH_3)_mH_{3-m}) = \Delta H^o(X^-CH_3^+) - m \psi_x + f^*(m) - \psi_x \ 
\]

Here \( f^*(m) = 27.91 + 2.38m + 11.07/m \).

For higher alkyl, we must include third, fourth, and higher order interactions between the formal charge and the paired electrons. As pointed out by Lossing and Holmes,\(^3\) the ionic charge is not localized at the formal charge site and must be distributed over the whole ion. We are inspired by the idea and give an approximate expression for the IPs of alkyl radicals

\[
\text{IP(alkyl)}_{\text{est}} = \text{IP}(C(CH_3)_mH_{3-m}) - 0.06 \gamma^+ \text{ eV} \quad (2)
\]

Here \( \gamma^+ \) is the total number of carbon atoms at and beyond the \( \gamma \)-position in the alkyl cation. The average deviation between the values estimated from eq. (2) and the observed values\(^4\) is 0.05 eV for 14 alkyl radicals.

For heats of formation of alkyl cations, we obtain

\[
\Delta_{f}H^o(\text{alkyl cation})_{\text{est}} = \Delta_{f}H^o(\text{alkyl radical})_{\text{est}} + \\
+ 23.06 \text{IP}(C(CH_3)_mH_{3-m}) - 1.4 \gamma^+ \text{ kcal/mol} \quad (3)
\]

Here heats of formation of alkyl radicals are estimated by well-known methods.
Benson's method. The average deviation from eq. (3) is 1.3 kcal/mol for 25 alkyl cations. For the heterolytic BDEs of X-alkyl bonds, we obtain

$$\text{DH}^o(X^-\text{-alkyl cation)}_{\text{est}} = \text{DH}^o(X^-\text{-C}^+(\text{CH}_3)_m\text{H}_3-m) - 1.4 \gamma^+ \quad (4)$$

The average deviation is 1.3 kcal/mol for 25 C-H bonds. Eq. (4) is valid for many substituents X, such as F, SiH$_3$, and so on, but the observed BDEs of many bonds are not available for comparison at present. The algorithms of eqs. (2) to (4) are so simple that one just counts the values of $\gamma^+$, if the values of IP(C(CH$_3)_m$H$_3-m$) and DH$^o$(X$^-$-C$^+(\text{CH}_3)_m\text{H}_3-m$) have been listed in the given Tables.

Lossing and Holmes found that the heterolytic BDEs of H-alkyl bonds are a linear function of the logarithms of the number of atoms in the alkyl cation. Beauchamp and co-workers found a qualitative correlation between the IPs of primary, secondary and tertiary alkyl radicals and the number of carbon atoms. The two groups, however, did not give an expression for estimating the heterolytic BDEs of H-alkyl bonds and the IPs of alkyl radicals. The qualitative and semi-quantitative descriptions of Drs. Holmes' and Beauchamp's groups have been quantified well in our work. The method used in this work has been extended to molecular cations, RX$^+$.

REFERENCES


Molecular Dynamics Simulations of Bulk Desorption
In Molecular Matrices.

Jan Sunner and Jentaie Shiea
Department of Chemistry
Montana State University

The nature of the desorption process(es) in Fast Atom Bombardment, SIMS, Plasma Desorption and Laser Desorption is a longstanding and extremely important problem. The FAB mechanism, for example, has been the subject of extensive experimental studies. Much has been learned about the complexity of the process; for example on the importance of surface activity, radiation-induced chemistry, gas-phase basicities, etc. to the mass spectra. However, it has been very difficult to obtain unambiguous results as to the nature of the fundamental desorption process.

In other areas of science, such as protein chemistry, liquid structure, solvation and phase transitions, Molecular Dynamics (MD) has proven essential for deepening our understanding. There is every reason to believe that this is possible also for desorption ionization. MD simulations by Garrison et al. have successfully explored the collision cascade regime in SIMS (1). We have embarked on a program to extend such calculations into the "thermal spike" regime. Such calculations should reveal the nature of the "bulk desorption process", that is likely responsible for a neutral yield of about 10^-3.

Previously, we have presented MD simulations of sputtering from 2-dimensional Lennard-Jones fluids (2). These calculations have now been extended to 3-d Lennard-Jones (LJ) fluids and to 2-d diatomics. Figure 1 shows a "snapshot" from a 3-d calculation consisting of 2250 LJ atoms with mass 40 amu and with a pair interaction energy of 1 kcal/mol. A 15x15x10 grid of such atoms were first equilibrated for 3 ps at ca. 2000 K and then for 2 ps at 300 K. During this time they were confined to a rectangular box of 35x46x46 A. The walls were "wetted" by introducing a 1 kcal/mol Lennard-Jones potential between the atoms and the walls. The wall pressures were monitored to ensure that they were close to 0 at 300 K. One ps before impact, the right hand wall was removed. A 130 amu projectile then hit from the right with a (low) kinetic energy of 100 eV. Figure 1, at 1 ps after impact, shows three high-kinetic energy atoms leaving the impact site and some "disturbance" of the surface, but does not reveal the processes inside. A good way to visualize these is to "cut out" a central section of the system. Such a sequence of snapshots is shown in Figure 2. The atoms are shaded according to the temperature calculated for the local region within a 10 A radius: white to 300 K; light gray to 800 K, dark gray to 2000 K, and black above 2000 K. Further, the arrows show the velocity of that same local group of atoms (atom radius = 100 m/s). It is seen that the pressure waves easily propagate to the edges of the system. In this calculation, they were reflected at the walls. The local high temperature at the impact site is seen to be rapidly quenched by energy transport throughout this small system. Figure 3 shows a system with a higher ratio of projectile impact energy to total bonding energy. In this cases, the system undergoes a bulk disintegration and it empties into space. However, allowing thermal transport through the walls tend to quench the thermal excitation such that no bulk desorption occurs. In FAB, the projectile energy is 100 times larger, and the rate of thermal quenching therefore should be much slower.

1) N. Winograd and B.J. Garrison, in Ion Spectroscopy for Surface Analysis, Czanderna and Hercules, Eds. Plenum, in press.
Fig. 1. 1 ps after impact on 2250 atom system.

Fig. 2. Sequence of snapshots from central slab in calculation in Figure 1. Times shown are 0.2, 1, 2, and 3 ps after impact.

Fig. 3. Bulk desorption of 1000 atom system.

Fig. 4. Naive picture of relation between present 100 eV calculation and typical 10 keV FAB process.
The MIKES spectrum of the [M-F] cation (Figure 1) of 1,1,2-trichloro-1,2,2-trifluoroethane (freon 113) exhibits a single peak that corresponds to a very curious fragmentation process that seems to involve migration of a Cl atom and subsequent formation of $\text{CCl}_3^+$ and the carbene $:\text{CF}_2$. The narrowness of the MIKES peak suggests that the formation of these two species involves a simple bond cleavage rather than a rearrangement. However, a rearrangement process prior to the C-C bond cleavage process is necessary. The MIKES spectrum of the [M-C1] cation (Figure 2) of freon 113 also contains a single narrow peak. However, this process can be rationalized as a simple bond cleavage. Semiempirical MO calculations have been used to complete a study of the energetics of the fragmentation processes observed in the metastable spectra of the [M-F] and [M-C1] cations. Similarly, the energetics of the various dissociation processes observed in the high-energy and low-energy CID spectra of the these two ions were also investigated for purposes of comparison with the metastable results.

The results of the semiempirical calculations concerning the low-energy processes observed in the metastable and low-energy CID spectra are shown in Figures 3 and 4. Figures 5 and 6 illustrate the results concerning the high-energy processes observed in the high-energy CID spectra, which all involve homolytic bond cleavages. The loss of the F atom bound to carbon one is favored over loss of one of the F atoms bound to carbon two by 8.5 kcal/mole during the formation of the [M-F] cation. Similar calculations for the [M-C1] cation show that loss of the Cl atom bound to carbon two is favored by only 3.3 kcal/mole. The barrier for isomerization of the higher energy form to the lower energy form is 28.8 kcal/mole for the [M-F] cation and 11.5 kcal/mole for the [M-C1] cation. A nearly symmetric transition state for Cl atom rearrangement was located for the lower energy form of the [M-F] cation. The energy barrier to this rearrangement is 8.8 kcal/mole. Overall, the semiempirical results show that the Cl atom rearrangement process is exothermic by 6.1 kcal/mole. The fragmentation of the rearranged ion to yield the $\text{CCl}_3^+$ and $:\text{CF}_2$ species is endothermic by 14.0 kcal/mole. A similar transition state was located for F atom rearrangement in the [M-C1] cation. The barrier to isomerization is 26.2 kcal/mole. However, it is not likely that a significant fraction of the ion population will isomerize because the enthalpy change for simple bond cleavage is only 15.7 kcal/mole. Simple bond cleavage of the respective higher energy forms of the [M-F] and [M-C1] cations is also possible; however, these are high-energy processes and are therefore only observed with low abundance in the high-energy CID spectra. Simple bond cleavage for the low-energy form of the [M-F] cation is also a high-energy process and is only present in the high-energy CID spectrum. The dissociation processes outlined in Figures 5 and 6 are all predicted to be high-energy homolytic bond cleavage processes and are, as expected, only observed in the high-energy CID spectra. The exceptional stability of the $:\text{CF}_2$ carbene compensates for large positive heats of formation of the methyl-type cations observed in metastable and low-energy CID mass spectra, thus making their appearance in these types of spectra possible. Similarly, the presence of the extra F atom in the [M-C1] ions increases their stability relative to those of the [M-F] ions. This would explain the fact that the electron ionization mass spectrum of freon 113 contains fragment ions that are mostly characteristic of the [M-C1] cation.

The bridging halogen atom in each of the transition states located in the study causes a shortening of the C-C bond in the transition state relative to that in the reactant ion, presumably by donating electron density to the carbon atoms. Not unexpectedly, the C-C bond order increases in parallel with the decreasing bond length in going from reactants to the transition states. It is also obvious that a bridging Cl atom produces lower energy transition states than a bridging F atom. One possible explanation of this is the higher electronegativity of the F atom.

Figure 1

![Metastable Spectrum: Methane Cl][M-F] → COCl+ + CF,

\[ T_{se} = 111.2 \text{ meV} \]

Figure 2

![Metastable Spectrum: Methane Cl][M-CI] → CCIF+ + CF,

\[ T_{se} = 30.6 \text{ meV} \]

Figure 3

![Potential Energy Surface][M-F]

Figure 4

![Potential Energy Surface][M-CI]

Figure 5

![Potential Energy Surface][M-F]

Figure 6

![Potential Energy Surface][M-CI]
The power of mass spectrometry as an analytical technique lies in the ability of the analyst to deduce the molecular structure of the compound under study from the data collected. Correlation analysis techniques (from information theory) can be applied to enhance the quantitative information present, as well as aid the analyst in the interpretation of low-resolution mass spectral data.

Although correlation analysis has been applied to data obtained from a variety of analytical techniques (including flame atomic fluorescence spectrometry (AFS), inductively-coupled plasma atomic emission spectrometry (ICP-AES), Fourier-transform infrared (FT-IR) spectrometry, UV/visible absorption spectrometry, multi-dimensional fluorescence spectroscopy, extended X-ray absorption fine-structure spectroscopy (EXAFS) and Mössbauer spectroscopy), its application to mass spectral data has been limited. This talk will demonstrate that the correlation procedure can be used to increase the discriminating power of the mass spectrometer (termed "compound specific detection") as well as aid in the determination of sequence information from peptide MS data.

**Compound Specific Detection:** In the early 1980's Anderegg described a technique called "isotope cluster chromatography", where the mass spectra obtained from a combined GC/MS analysis are analyzed automatically to identify those chromatographic peaks containing one of a number of different distinct isotope patterns (such as chlorine or bromine). While the correlation analysis technique can be used for such "substructure specific" detection, it is demonstrated here that this idea can be extended to enable the mass spectrometer/data analysis system to serve as a compound specific detector. This is accomplished by cross-correlating each mass spectrum obtained from the instrument with a library spectrum of the compound of interest. This is demonstrated using the GC/laser ionization TOFMS data for a series of alkyl-benzenes plus naphthalene presented by Opsal and Reilly.

**Neutral Loss Spectrum:** Crawford and Morrison noted that for many types of compounds, such as those containing a long aliphatic chain, that prominent peaks occur at periodic spacings on the mass axis. They suggested that a "mass periodicity spectrum" or "neutral loss spectrum" could be calculated from the raw data, and that the process is similar to auto-correlation. In fact, the auto-correlation function calculated from a mass spectrum is composed of two copies of the neutral loss spectrum, i.e., they consist of all those peaks at tau values other than zero. For example, any pairs of peaks in the original mass spectrum separated by 14 mass units will contribute intensity to the position in the auto-correlation function corresponding to a mass loss of 14. We would expect that different compounds of the same chemical class would show similar neutral loss spectra. Making use of this fact, a number of workers, have successfully used the auto-correlation procedure as a pre-processing step in chemical pattern recognition studies.

It is demonstrated here that neutral loss data (obtained directly from the auto-correlation procedure) may also aid in the elucidation of sequence information from the analysis of peptide MS data. This is demonstrated below in figures 1 and 2. Figure 1 shows the auto-correlation of the infrared laser desorption/UV laser post-ionization mass spectrum of the penta-peptide met-enkephaline (Tyr-Gly-Gly-Phe-Met) reported by Grotemeyer. The neutral loss spectrum corresponding to the positive side of the auto-correlation function, including all mass losses between 1 and 250 amu, is reproduced in detail in figure 2. Although there are a number of peaks due to non-structurally significant fragmentation, peaks corresponding to the residue mass of the amino acids glycine, methionine, tyrosine and phenylalanine are clearly visible. In addition, neutral losses corresponding to the dimers Gly-Gly and Gly-Phe are also observed. Most importantly, identification of the peaks in the neutral loss spectrum can be easily automated by cross-correlating the neutral loss spectrum with synthesized data class spectra, using the correlation procedure as a substructure specific detector.

References:
**The 39th ASMS Conference on Mass Spectrometry and Allied Topics**


TOWARDS AN INTUITIVE DATA SYSTEM FOR MASS SPECTROMETRY

P. R. Bates, M. S. Henderson, M. P. Stevenson, J. S. Cottrell,
Finnigan MAT Ltd., Hemel Hempstead, Herts., UK.

Introduction

A Microsoft Windows 3.0 based data system has been written to provide instrument control and data processing facilities for a new laser desorption time-of-flight mass spectrometer. The key design goal was ease of use.

Instrument Control

Instrument control is achieved through the use of a window containing a mimic diagram. Because only a few instrument parameters need to be adjusted, the control window can be kept relatively simple.

Viewing data

The data window contains the spectrum trace. Rescaling the display can be performed using menu commands or by dragging out an area with the mouse. A barbell which functions like a sophisticated scrollbar is also available.

Peak detection

If peak detection parameters need adjustment, a toolbox allows the user to define model peaks and model baseline segments. This information is then used by the software to refine the peak detection parameters.

Calibration

Time to mass conversion is performed automatically using two stored calibration constants. If reference peaks of known mass are present in the spectrum, the user may identify them graphically and obtain a more accurate internal calibration.
Reports

Peak detection generates a tabular report listing of peak masses and areas. Reports may be formatted to include any other system parameters or sample information. A report template editor provides limited desktop publishing facilities for customising report layout and content.

Multiple Samples

Multiple samples may be analysed or multiple data files reprocessed using a spreadsheet-like Sequence table. Each row represents a sample and each column a logical group of parameters controlling system operation.

Online Help

Online help is always available and provides assistance with operating procedures, commands, and terminology. The help system is context sensitive, taking the user directly to information relevant to the current display or action.

Data Exchange

One of the benefits of running under Windows 3.0 is the simplicity with which numerical and graphical data may be interchanged between applications. For example, it is possible to transfer data dynamically to a Microsoft Excel spreadsheet and perform a real time statistical analysis of the mass measurements.

Conclusion

An intuitive, graphical approach to instrument control and data processing has been adopted. Peak detection and calibration are simplified through the use of graphical toolboxes. Analysis of multiple samples uses a spreadsheet architecture. Data exchange to third party applications effectively extends the functionality of the software to include the enormous number of Windows based applications.
SOFTWARE PROGRAMS FOR PROCESSING
PCDF/PCDD GC/MS DATA

*David S. Weinberg, R. E. Adams, and M. Lisa Manier
Southern Research Institute
Birmingham, AL 35205

A. Target PCDFs/PCDDs

VG GC/MS PCDF/PCDD data are produced by window mix, initial calibration (ICAL), continuing calibration (CCAL), blank, sample, matrix-spike (MS), and matrix-spike-duplicate (MSD) runs. We initiate the processing of the acquired data on the VG 11-250J data system by using user-prepared RUN programs that employ VG-provided peak detection (PKD), peak report (PKR), peak quantification (PKQ), and report generation (RPG) programs and various user-written macro (MAC) programs.

We then further process the files on a microcomputer (PC) by using a user-interactive batch file named RUNZ.BAT (the "Z" in "RUNZ" is the number of LOTUS 123 spreadsheet files ultimately to be processed in the batch). While using the batch file, we employ a KERMIT protocol to transmit the ASCII report (RPT) files located in the VG RSX [70,42] directory to the C:\KERMITV subdirectory in the PC.

We then individually import the files into appropriate LOTUS 123 spreadsheets and use LOTUS 123 macros to perform calculations. (Three separate worksheets formats are used: one for an ICAL; one for a CCAL; and one for a sample, a blank, an MS, or an MSD). The worksheet files are then sent to the C:\DBXL subdirectory and are further processed by a DBXL database program named LOTUSZ (the "Z" in "LOTUSZ" is the same as defined above.) The newly created database files are saved as text files and are copied to the C:\KERMITV subdirectory. We then use a KERMIT protocol to transmit the files to the [DWEINBERG] subdirectory on the DEC VAX 8550 minicomputer and complete the preparation of reports using a SAS program named MENUDISP.SAS.

B. Total PCDFs/PCDDs

As mentioned above, we initiate the processing of the acquired data on the VG 11-250J data system using user-written RUN programs that employ VG-provided peak detection (PKD), peak report (PKR), peak quantification (PKQ), and report generation (RPG) programs and various user-written macro (MAC) programs. However, we determine total PCDFs and PCDDs only in blanks and samples. To that end, we further process only the ASCII files with the generic name VOXXXXS.RPT generated by those programs for blanks and samples. We also use a second RPG program on the same PKS files previously generated by the PKD program to produce a second type of ASCII file with the generic name VOXXXXSS.RPT.

We mentioned above that we further process the target-compound files on a microcomputer (PC) using a user-interactive batch file named RUNZ.BAT (the "Z" in "RUNZ" is the number of LOTUS 123 spreadsheet files ultimately to be processed in the batch). We indicated that ultimately we produce files with the generic name VOXXXXS.TXT, and we transfer them to the C:\KERMITV subdirectory. We use those same files generated for blanks and samples for the determination of total PCDFs and PCDDs. Consequently, we copy such files to the C:\LP77 subdirectory for final processing.

We process the files with the generic name VOXXXXSS.RPT using a user-interactive batch file named TOTZ.BAT. (Note: "Z" in "TOTZ" is the same as defined above.) While using the TOTZ.BAT batch file, we employ a KERMIT protocol to transfer the ASCII file VOXXXXSS.RPT to the KERMITV subdirectory. We then individually incorporate each such RPT file into a "TOTALS" spreadsheet in LOTUS 123 and then separately save each worksheet file. We copy each file to DBXL, use a DBXL program named TOTZ.PRG (Note: the "Z" in "TOTZ" is the same as defined above) to further process the data, and then separately save each processed file again. Then we transfer each of these files with the generic name VOXXXXS.TXT to the LP77 subdirectory for final processing along with the VOXXXXS.TXT files. We use a Fortran 77 program named "TOTALSF.FOR" to produce and print a final report for each blank or sample.
### ISOTOPE DILUTION CALCULATED CONCENTRATIONS

#### SAMPLE

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<th>Run</th>
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<th>Retention time (s)</th>
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<th>RRF</th>
<th>Amount (ng)</th>
<th>Concentration (ng/kg)</th>
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<td>2,3,7,8-TCDF (H)</td>
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### TOTAL TCDFS AND TCDDs

**GC/MS Run No. V0399055**

Sample dry mass = 0.01000 kg

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<th>Average RRF</th>
<th>Amount (ng)</th>
<th>Area (ng)</th>
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THE USE OF DYNAMICALLY PROGRAMMED SCANS TO GENERATE MS/MS/MS SPECTRA WITH AN ITMS®

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The ion trap mass spectrometer (ITMS®) allows successive stages of mass spectrometry to be performed within the same electrode structure [1]. A second useful feature of the instrument is contained within the controlling software, which allows key sequence files to be written which can then be incorporated into the data system. One application of this methodology has been proven in the automatic recording of tandem mass spectral data, where the need for a time-consuming setting up procedure has been obviated. By varying a parameter which permits fine tuning of the frequency of the excitation voltage (the DELTA-FREQUENCY), one can compensate for the discrepancy between the calculated and observed values of the resonant excitation frequency [2]. In this communication we report upon the application of techniques developed in this laboratory to the study of MS/MS/MS spectra of some simple compounds acquired under the control of dynamically programmed scans (DPS) [2] to yield 'familial' spectra of the type reported with a pentaquadrupole instrument [3].

A major difference between collision induced dissociation in the ion trap and in ion beam transport instruments, such as the triple- and pentaquadrupoles, is that in the former, one has to specifically tune each mass which is to be resonantly excited ('tickled') in order to ensure that collisional excitation always occurs at the same value of the parameter \( q \). This is equivalent to ensuring that the in the quadrupole the collision energy is held constant. It is therefore necessary to know which masses are to be tickled during the experiment before the tickling process is attempted. As an example, the operational sequence for a 'fixed parent ion' scan is shown in Fig. 1. Here the aim is to identify what daughter ions are formed from a single parent, and then acquire the grand-daughter mass spectrum for each of these daughter species (expressed as \( \bullet - O - O \) in the symbolism employed by Cooks and co-workers [3]). The operator first selects one or more possible parent ions which are to be studied on the basis of prior knowledge about the compound (or this could be done using DPS), after which the MS/MS/MS scan function for the experiment is loaded by the computer; note that some of the tickling parameters such as the TICKLE TIME, TICKLE VOLTS, the \( q \)-value and the DELTA-FREQUENCY have already been optimised and set in the scan function. The first selected parent ion, is then tickled in a 'pre-scan' in order to discover its daughter products and this information is read to a file. Each of these daughter ions is then selected in turn and tickled so as the generate the grand-daughter spectrum. Note that because the ion trap does not provide any external read-out to indicate which masses are being tickled it has been necessary to devise an 'internal' indicator. Thus for each daughter ion which has been discovered in the pre-scan, a sequence of three groups of ten spectra (which may then be averaged) are acquired, the parent-only, the daughter-only and the grand-daughter spectra (see Fig. 1). The resulting data takes the form of the 'fragmentogram' for the possible parent ions in 1,2-dichlorobenzene shown in Fig. 2, and which may be manipulated using the standard ITMS® software, or transferred to other programs which permit further refinement and display by means of graph-plotting software. An example of a partial 'familial' scan in which the grand-daughter spectra resulting from \( m/z 55 \) as a fixed daughter ion are plotted against the various parent \( m/z \)-values (\( O - \bullet - O \)) is shown in Fig. 3.

From this work we conclude that (1) it is possible to automate the acquisition of MS/MS/MS spectral data on the ITMS; (2) using the sequences outlined here it is possible to acquire data matrices from which different forms of tandem mass spectral display may be constructed; (3) the work points the way to the development of semi-intelligent compound identification and/or structure determination using multiple stages of sequential mass spectrometry.

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References.


Acknowledgements.

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The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Specify parent ions?

Load scan function

A B C D Scans

(a) on off off off 1 - 10
(b) on on on off 11 - 20
(c) on on on on 21 - 30

Selects parent, reads daughter spectrum to file: A on B on C off D off

Repeat for next daughter

Figure 1

FAMILIAL SCAN, M/Z 55 FROM n-DECANE

Figure 2

Figure 3

525
Electrospray Combined with an Ion Trap Mass Spectrometer for the Determination of Neuropeptides

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Introduction

Mass spectrometry methods for the detection and identification of neuropeptides in plasma or cerebrospinal fluid at physiological levels requires specificity and sensitivity exceeding that obtained from RIA methods. A mass spectrometric approach for neuropeptides which is potentially very sensitive and specific is the combination of electrospray with an ion trap mass spectrometer (ITMS). The electrospray technique offers the compatibility with HPLC separation and high ionization efficiencies for peptides in the 10-100 molecular weight range. The ITMS offers increased sensitivity over quadrupole instruments, it operates at higher pressure than many mass spectrometers making it ideal for HPLC coupling, and it has the ability to perform MS^n experiments for a fraction of the cost for most tandem MS instruments. This paper reports on the coupling of a commercial electrospray interface to an ITMS and the optimization of its performance for the detection of neuropeptides.

Experimental

Samples dissolved in 50% methanol in water containing either 0.1% trifluoroacetic acid or 3% acetic acid in the concentration range of 100 ng/mL to 0.01 ng/mL were introduced into the electrospray interface (Vestec Co.) 2 μL/min. The electrospray interface was operated with a needle voltage of 2.2-2.4 KV, nozzle voltage of 200 V, repeller voltage ranging from 5-80 V, source voltage of 4.5 V and lens voltages (lens 1 and 3) of -70 V. A gate was added to the electrospray interface (lens 2) to pulse ions into the ion trap at specific time intervals ranging from the 10-200 ms. The gate was controlled by the existing ±180 V circuit to gate electrons for EI operation. The gate voltages from the ITMS were conditioned so that potentials ranging from -100 to 0 V could be selected to eject ion into the trap and potentials from 12 to 100 V could be selected to prevent the entrance of ions into the trap. The electrospray interface was mounted into the existing ITMS filament end cap with a self-centering lens assembly to insure the electrospray and end cap aperture were in alignment. The electrospray-ITMS assembly was mounted on the opposite end of the vacuum chamber relative to the normal EI operation position to minimize hardware changes that would be required for the coupling.

The ITMS was operated under standard conditions for ions in the mass range of 0-650 daltons. Helium at an Ionization gauge reading of 2 x 10^-5 torr was added to the ITMS (total pressure with helium and electrospray in operations was 4-5 x 10^-4 torr) to stop the ions gated in the trap. For higher molecular weight peptides, the trap was operated in the resonance ejection mode to increase the mass range of the instrument to 2600 daltons.

Results and Discussion

The signal from the combination of electrospray with the ITMS was evaluated as a function of parameters that effect ion transmission into the ion trap. These parameters included gating voltage, RF voltage on the ring electrode, position and voltage differential of the nozzle relative to the skimmer and helium pressure in the trap. The gating voltage (Figure 1) optimization indicated that between -40 to -150 V was best for ejection of ions into the trap. Higher negative voltages resulted in collisional activation decomposition (CAD) of the sample or ions that could not be stopped inside the trap. Ions were best gated from entering the trap at potentials greater than 50 V. The RF voltage had some effect on signal intensity (Figure 2), with lower RF voltages showing the best signals for low molecular weight compounds. Compounds with ions above m/z 500 could be analyzed at higher RF voltages (mass values of 50-100). The nozzle and skimmer position (Figure 3) and voltage (Figure 4) played an important role in transmission and in CAD of the ions generated by electrospray. The best signal was obtained with the skimmer extended 3.5 turns (2.79 mm) from the full inserted position. At this position the foreline vacuum reading of 0.69 torr. The voltage on the skimmer can be used to CA the ions formed by electrospray to generate daughter ions. Typically for low molecular weight compounds skimmer potentials of 5-15 V resulted in minimal fragmentation while skimmer potentials greater than 30 V resulting in significant fragmentation. The last parameter, helium pressure in the ion trap, (Figure 5) indicated that helium was necessary to stop the ejected ion formed in electrospray. With no helium (pressure 1 x 10^-4 torr) the electrospray ITMS signal was weak and very noisy. The addition of 2-4 x 10^-4 torr helium (uncorrected ion gauge readings, actual pressure of helium is 12-15 times higher than the gauge reading) greatly increased the signal level recorded, since the ejected ion now could be trapped.
Neuropeptides ranging from 5 (leucine enkephalin) to 31 amino acids (β-endorphin) shown in Figure 6 were determined by electrospray ITMS. The electrospray spectra showed primarily single and multiple charged protonated molecular ions. Full scan mass spectra (scanning from 0-2600 daltons) could be recorded on as little as 10 femtols of β-endorphin. The electrospray ITMS combination was slightly more sensitive than the equivalent determination of β-endorphin on a quadrupole instrument; however, further optimization of the electrospray ITMS is required and significant improvements in sensitivity are anticipated.

Acknowledgements

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CHARGE LIMITED SCALING RELATIONS FOR THE ION TRAP DETECTOR

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INTRODUCTION

The product of detection sensitivity and resolution $R \sim m/\delta m$ may often be simply expressed. The resolution of resonant ion ejection in the Ion Trap Detector[1] is reduced by anharmonic perturbation of electric fields produced by trapped charge. Sensitivity is proportional to the amount of trapped charge. The amount of charge is proportional to the cube of the cloud radius. Previous work has assumed that cloud radius is equal to the size of the trap. With collisional damping we take this radius as a free parameter.

In the pseudopotential approximation expounded by Dehmelt[2], ions are trapped in a harmonic potential well created by the gradient of the of the energy density of the quadrupole field, $d^2r/dt^2 = -(1/4\Omega^2) \nabla(\epsilon E/m)$. For convenience we consider a spherical pseudopotential of radius $a$. A test ion of mass $m_j$ and charge $e$ oscillates with a frequency $\omega_j$. $\omega_j = q_j\Omega/2\sqrt{2}$, where $q_j = 4eV/m_ja^2\Omega^2$. $V$ is the trap AC voltage at frequency $\Omega$. With helium damping, ions collect in a cloud at the center of the trap, in which space charge forces are balanced by the pseudofield.

The trap may be filled with ions of mass $m_0$ to a radius $r_o$. If $Q$ is the trapped charge, we find that $Q = (\pi e Om_0/2e)q_0^2\Omega^2r_o^3$. The charge density is independent of $Q$, and as ions are added $r_o$ increases. $Q$ is proportional to $1/m_0$. If we add a set of heavier ions, the force balance requires that these collect in an outer shell, separated from the inner sphere. For a single heavier ion within the cloud, the pseudofield is weaker and it is pushed outward by space charge fields to an equilibrium radius $r_i > r_o$. As more of the same heavy ions are added, the shell grows outward from $r_i$ with constant but lower charge density according to the heavier ion mass.

Now we consider the effect of $Q$ on a lighter test ion of mass $m_j$. The resolution of resonant ejection of the test ion will be degraded by the electric field of the cloud. When the trap voltage is ramped (increased) the test ion will be ejected first while the cloud is stable. The corresponding experiment is to fill the trap to a level $Q$ from a heavy gas containing a light tracer which is close to its point of extraction. The limit on $Q$ will determine the least concentration of tracer that can be detected. We will stop with determination of $Q$. The test ion moves in a field that is the sum of the pseudofield and the space charge field. The radial, free motion $z_j$ of the test ion is given by $d^2z_j/dt^2 = -\omega_j^2(z_j - (m_j/m_0)r_o^2/z_j^2)$ for $z_j > r_o$, $d^2z_j/dt^2 = -\omega_j^2z_j(1 - m_j/m_0)$ for $z_j < r_o$, where $\omega_j$ is the pseudopotential frequency. When $m_j = m_0$ the force is zero inside the cloud. For resonant excitation the period of motion matches the
period of excitation for all amplitudes $z_j$. But the period $\tau$ varies in the range $0 < z_j < a$, and we conclude (essentially) that
\[
\Delta \tau / \tau = (m_j / m_0) (r_0 / a)^3 = 1 / R
\]
where $R$ is the limiting resolution. We have also considered the self motion of the spherical cloud due to the image charge produced on the trap electrodes[5], with a similar result ($m_j = m_0$).

Thus we get the following $QR$ product
\[
QR = 4\pi\varepsilon_0 q_j aV = \pi\varepsilon_0 (m_j / e) q_j^2 \Omega^2 a^3.
\]
These expressions are related by the definition of $q_j$. For a given operating point $q_j$, the first expression is independent of mass, showing that $QR$ is proportional to the product of the trap radius and the applied AC voltage. For typical trap parameters at $R=1000$, we get $Q/e \approx 10^7$ ions, and $r_0 = 1$ millimeter.

While this result is strictly valid within the mathematical range of the pseudopotential approximation, i.e. for $q_j < 0.4$, we propose that it is likely to be valid out to the stability boundary at $q_j = .908$. When the secular orbit frequency $\omega$, increases to half the applied frequency, the orbit becomes unstable by the universal mechanism of parametric excitation. Of course, it is unusual that the pseudopotential and the parametric pump are produced by the same field. But the the sharpness of the boundary is direct proof of the existence of a harmonic potential well at the $\Omega/2$ resonant orbit frequency. In this case, the driving field at frequency $\Omega$ is a parametric axial modulation.

In future work this zeroth order model will be extended to include the case of finite ion temperature. In this case the charge number density $n$ is assumed to be determined by Boltzmann statistics, viz.
\[
\ln(n) = -(1 / kT) \int (m\omega^2 r - eQ(r) / 4\pi\varepsilon_0 r^2) dr, \quad \text{where} \quad dQ / dr = 4\pi\varepsilon_0 n r^2.
\]
The effective potential in the exponential factor is the sum of the RF pseudopotential and the potential of the trapped charge.

I am indebted to Dr. S.E. Buttrill, Jr. at Varian Assoc. for his interest, and to Dr. N.J. Kirchner for pointing out to me that trapped ions are configured as shells.

Implementation of Parent and Neutral Loss Scanning and Selected Reaction Monitoring on a Quadrupole Ion Trap Mass Spectrometer

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MS/MS parent and neutral loss scanning and selected reaction monitoring (SRM) are easily implemented on tandem-in-space MS/MS instruments (e.g., triple quadrupoles), but until recently have not been implemented on tandem-in-time MS/MS instruments (e.g., quadrupole ion trap mass spectrometers, ITMS). We have developed two different methods to implement these scan modes on the ITMS. Although the parent scan and SRM methods are described below, the principles are similar for a neutral loss scan.

Both methods of implementing parent scanning rely on the fundamental principle that for a given set of instrumental parameters (largely RF voltage), each ion will have a unique secular frequency dependent upon its m/z. Resonant excitation at a frequency equal to the secular frequency of an ion of interest can be used to promote either collisionally induced dissociation (CID) or resonant ejection of that ion from the ion trap to the detector.

The first method for implementing a parent scan involves the simultaneous application of two resonant excitation waveforms during the CID period of a normal ITMS MS/MS scan function (Figure 1). The frequency of one of the resonant excitation waveforms is scanned at low voltage to induce CID of successive parent ions. The other waveform is applied at the secular frequency of the daughter ion of interest and at a high enough voltage to cause rapid resonant ejection. When CID of a parent ion produces the desired daughter ion, it is ejected from the ion trap and detected. Figure 2 illustrates the result of such an experiment for the parents of 91+ for a mixture of n-alkylbenzenes. There are a number of disadvantages to this technique, most markedly the ejection of parent ions (as noted in Figure 2B) and the ejection of daughter ions with q>0.91; both can result in high estimates of the parent ion intensities.

A second method was devised to overcome the shortcomings of simultaneous resonant excitation and ejection. In this method the parent ion and daughter ion resonant excitation waveforms are applied sequentially in time, or pulsed. First CID of the parent ion occurs, then rapid ejection of the daughter ion of interest, then CID of the next parent ion, etc. Figure 3 illustrates this method for a selected reaction monitoring (SRM) parent scan of 91+ for a mixture of n-alkylbenzenes where only the

![Figure 1. Simultaneous resonant excitation parent scan function.](image1.png)

![Figure 2. Simultaneous resonant excitation parent scan of 91+ of n-alkylbenzenes.](image2.png)
expected M$^+$ ions are scanned. Figure 4 illustrates an expanded version of two pulse sequences having different parent ion resonant excitation voltages. As can be seen in these figures, if any parent ion is ejected or if any daughter ions with q>0.91 are formed and ejected during the parent CID pulse, their ion signals will not interfere with the ion signal due to the daughter ion of interest. In addition, the parent ion resonant excitation voltage can be optimized for the parent m/z range of interest. Of course, for SRM, it is straightforward to alternate between any parent and daughter ion combination of interest.

![Figure 3. Pulsed SRM parent scan of 91$^+$ for a mixture of n-alkylbenzenes.](image)

![Figure 4. Two pulse sequences expanded from pulsed SRM parent scans of 91$^+$ for a mixture of n-alkylbenzenes.](image)

We have not yet demonstrated neutral loss scanning experimentally. However, its implementation will be similar to the pulsed parent scan described above. With a neutral loss scan, the daughter and parent masses must be scanned. As such, two different methods can be implemented depending upon the behavior of the ring RF voltage. If a constant ring RF voltage is maintained, the resonant excitation frequencies of the daughter ion and parent ion can be scanned (in a pulsed method) as indicated in Figure 5. Alternatively, if the ring RF voltage is scanned in a linear fashion to maintain a constant daughter ion resonant excitation frequency, then the parent ion resonant excitation frequency can be scanned as illustrated in Figure 6. The latter method would yield better parent ion and daughter ion mass resolution and a greater applicable m/z range yielding more efficient CID.

![Figure 5. Secular frequencies of parent and daughter ions and the ring RF voltage for a neutral loss scan at a constant RF voltage.](image)

![Figure 6. Secular frequencies of the parent and daughter ions and the ring RF voltage for a neutral loss scan with a constant daughter ion q.](image)

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ENHANCEMENT OF MASS RESOLUTION IN THE QUADRUPOLE ION TRAP VIA RESONANCE EJECTION

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Some of the attributes of the quadripole ion trap include high MS/MS efficiency, the capability for multiple stages of mass spectrometry (MS^n), high sensitivity, the capability for ion-molecule reactions, and relatively low cost. However, the Finnigan MAT ion trap mass spectrometer (ITMS) has unit mass resolution and a nominal upper mass/charge limit of only 650 when operated in the standard mass-selective instability method for acquiring mass spectra. Mass resolution up to ~ 2500 can be achieved with the use of the axial modulation technique. Ions can also be ejected at a q_e value other than that corresponding to the mass-selective instability boundary by application of an auxiliary signal to the endcap electrodes. When the frequency of this signal is resonant with the secular frequency for a particular m/z, such ions can become kinetically excited and ejected from the trap via a process known as resonance ejection.

Last year, we demonstrated the capability to mass-selectively isolate or eject ions\(^1\) over a wide mass range and to generate mass spectra of electrospray-generated ions by scanning the frequency of the resonance ejection signal applied to the end-cap electrodes\(^2\). A block diagram of the experimental modifications to the ITMS\(^\text{TM}\) required for frequency-swept resonance ejection is shown in Figure 1. The mass resolution of the ion trap can be further enhanced by slowing the frequency scan rate and reducing the amplitude of the resonance ejection signal. El mass spectral data acquired for the m/z 502 ion from perfluorotributylamine (PFTBA) acquired with axial modulation (Figure 2a) and frequency-swept resonance ejection (Figure 2b) are shown. The low resolution spectrum (m/Am = 1900) was obtained at a scan rate of 5555 m/z per second, while the high resolution spectrum (m/Am = 45,000) was acquired at 4 m/z per second. Although the amplitude of the m/z 502 signal from mass-selective instability is approximately fifty times greater than from resonance ejection, the corresponding peak width in time units is about fifty times less. Thus, the areas under the two peaks, which correspond to the total number of ions at m/z 502, are the same within experimental error. The mass resolution is shown in Figure 3 as a function of scan rate at m/z 502, q_e=0.85. The mass resolution is also a function of the q_e and m/z values for a particular ion as well as the frequency scan rate. Plots of the mass resolution versus these parameters are shown in Figure 4, for m/z 502, and in Figure 5, for q_e=0.85. Each data set was obtained at a sweep rate of 1.6 m/z per second.

Fig. 2a

Intensity (Thousands)

\( \Delta m = 68 \mu s \)

\( m/\Delta m = 1900 \)

m/z

501.50 502.00 502.50 503.00 503.50

Fig. 2b

Intensity (Thousands)

\( \Delta m = 2.8 \mu s \)

\( m/\Delta m = 46000 \)

m/z

501.50 502.00 502.50 503.00 503.50

Fig. 3

\( q_i = 0.85, m/z = 502 \)

Scan Rate (Da/s)

Fig. 4

\( m/z = 502, 1.6 \text{ Da/s} \)

Fig. 5

\( q_i = 0.85, 1.6 \text{ Da/s} \)

m/z

CHEMICAL CHARACTERIZATION OF MICROPARTICLES BY LASER ABLATION IN AN ION TRAP MASS SPECTROMETER

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We are developing a new technique for the chemical characterization of microparticles based upon the use of electrodynamic traps. The electrodynamic trap has achieved widespread use in the mass spectrometry community in the form of the ion trap mass spectrometer or quadrupole ion trap (1). Small macroscopic particles (microparticles) can be confined or levitated within the electrode structure of a three-dimensional quadrupole electrodynamic trap in the same way as fundamental charges or molecular ions by using a combination of ac and dc potentials (2). Our concept is to use the same electrode structure to perform both microparticle levitation and ion trapping/mass analysis. The microparticle will first be trapped and spatially stabilized within the trap for characterization by optical probes, i.e., absorption, fluorescence, or Raman spectroscopy. (We have previously shown that such spectroscopic probes can be extremely sensitive, e.g., a detection limit of one molecule of Rhodamine-6G has been determined in the case of fluorescence spectroscopy (3), (4)). After the particle has been optically characterized, it is further characterized using mass spectrometry. Ions are generated from the particle surface using laser ablation or desorption. The characteristics of the applied voltages are changed to trap the ions formed by the laser with the ions subsequently mass analyzed. The work described here focuses on the ability to perform laser desorption experiments on microparticles contained within the ion trap. Laser desorption has previously been demonstrated in ion trap devices by applying the sample to a probe which is inserted so as to place the sample at the surface of the ring electrode (5), (6). Our technique requires the placement of a microparticle in the center of the trap. Our initial experiments have been performed on falling microparticles rather than levitated particles to eliminate voltage switching requirements when changing from particle to ion trapping modes.

Figure 1 shows a schematic diagram of our current apparatus for performing these experiments. The ion trapping device is a modified Finnigan MAT Model 800 Ion Trap Detector (ITD). The trapping electrodes were removed from the ITD vacuum chamber and placed in a 6-inch cube with the rotational symmetry axis of the trap oriented vertically. The cube is attached to a 4-inch oil diffusion pump. The rf voltage from the ITD was reattached to the ring electrode by extending the transformer tap wire to a high-voltage vacuum feed-through mounted on one of the side ports and retuning the transformer for resonance. The ITD was operated with nominally 1 mtorr of He buffer gas as usual. The ion lens assembly normally used for electron-impact ionization was removed from the top end cap and a particle dropping device was installed. The particle dropper consisted of a funnel-shaped container with a 450-μm spout. A 300-μm wire attached to a 6-mm rod normally rests in the spout. The rod exits the vacuum chamber through an o-ring sealed fitting. Particles of interest are placed in the container and dispensed by moving the wire attached to the rod. The other three side ports were fitted with 6-inch pyrex windows while the bottom port was covered with a flange mounted with the channeltron electron multiplier. A 5-mW HeNe laser beam is focused into the trap through the windows and opposing 3-mm holes drilled in the ring electrode. The HeNe beam is positioned ≈ 1 mm above the center of the trap. The second harmonic laser radiation from a pulsed Nd:YAG laser
(Quanta-Ray DCR-2A) propagates in the opposite direction through the trap and is focused by a 1-m lens at the trap center.

Collection of a laser desorption mass spectrum requires synchronization of the Nd:YAG laser with the falling particle and the ITD. The ITD runs continuously through its normal scan sequence of trapping and mass-selective particle ejection. The ITD can have a trapping-mode duty cycle of \( \approx 40\% \) if appropriate scan settings are used. When particles are dropped they pass through the HeNe probe beam scattering light that is detected by the photodiode. The photodiode signal is converted to TTL, then ANDED with the electron gate signal from the ITD. If a light scattering signal is detected when the ITD is in the trapping mode, the Nd:YAG laser is fired after an adjustable delay time. The laser trigger delay allows for the spatial displacement between the HeNe and Nd:YAG beams. Particles can be reliably illuminated with the 10-ns pulse from the Nd:YAG laser after proper alignment.

Silicon carbide particles (nominal 125-µm diameter) coated with various materials were used in our initial studies. Particles were coated with various quaternary ammonium or phosphonium salts by dissolving them in methanol, combining with a given mass of particles and evaporating the solvent. In all cases 5 mg of salt was evaporated onto 5 gm of particles. This loading would result in roughly 10 monolayers of material on the surface of a particle based upon particle surface area measurements. Compounds investigated include trimethylphenylammonium chloride, triethylphenylammonium iodide, tetrabutylammonium iodide, and tetraphenylphosphonium bromide. All experiments were performed with pulse energies of \( \approx 1 \text{mJ} \) (\( 10^9 \text{W/cm}^2 \)) except where noted. Ions were reliably produced from dropped particles with yield correlating with the intensity of the 532-nm light scattered by the particle as detected by the photodiode. The quality of the mass spectra varied primarily due to what appears to be space charge effects. Mass spectra of the above compounds all produced intact cations and expected fragment ions. The mass spectra compare favorably with those of Ref. 6 and SIMS data on the same particles.

In addition to the above coated SiC particles, uncoated particles of SiC, Fe, and Nb were also investigated. The uncoated SiC particles yielded Na and K ions presumably due to surface contamination. No ions were observed that would be associated with SiC even at intensities of \( 10^8 \text{W/cm}^2 \). The iron and niobium particles required slightly higher energies (\( \approx 3 \text{mJ/pulse} \)) to yield ions as would be expected. The iron spectra included the iron isotopes in addition to showing a copper impurity. The Nb spectra also indicated some iron contamination.

Improvements in the current apparatus promise to yield good sensitivity for materials on the surface of microparticles. Submonolayer sensitivities should easily be achieved. Excess ion production leading to space charge effects in the trap is currently more of a problem than lack of signal. Combining particle levitation with ion trapping may allow multiple desorption experiments to be performed on a single particle and thus permit signal averaging.

ION ACTIVATION IN A QUADRUPOLE ION TRAP

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Collision-induced dissociation (CID) can be performed in a quadrupole ion trap by resonantly exciting ions at a given mass-to-charge ratio so that they will undergo energetic collisions with the helium bath gas. While this is often compared to CID in a triple quadrupole or hybrid mass spectrometer, there are important differences. One of these differences is that once the parent ion dissociates in the quadrupole ion trap the product ions are not in resonance with the excitation voltage and thus are unlikely to undergo energetic enough collisions to cause further fragmentation. This is in contrast to beam instruments in which, under multiple collision conditions, product ions can be subsequently energized and dissociated by collision after they are formed. Another difference is that the parent ion is constantly accelerated by the resonance voltage in the quadrupole ion trap and thus, after an inelastic collision in which the ion loses kinetic energy, the next collision can be even more energetic because of the acceleration that occurs between collisions. Additional, the parent ions in the MS/MS ion trap experiment can undergo hundreds of collisions because the time-scale and path length of the CID portion of the experiment are orders of magnitude greater than in beam instruments.

In our effort to obtain a better understanding of the CID process in the quadrupole ion trap we have been studying several model compounds (in this discussion the compound studied was N,N-dimethylaniline) to determine the effects of the various parameters such as resonant voltage amplitude and duration, fundamental rf amplitude, and target gas pressure and composition. The resonant voltage amplitude and duration are factors in the power absorption of the resonated ions according to equation 1 below

\[ A = e^2E_0^{2}\frac{t_0}{8m} + \frac{\text{d}v/\text{d}t}_{\text{collide}} \]  

in which \( E_0 \) is the resonant electric field amplitude, \( t_0 \) is the duration of the resonant excitation and \( \frac{\text{d}v/\text{d}t}_{\text{collide}} \) is representative of all the collision processes that can affect the energy of an ion. (It should be noted that the first term in equation 1 is dependant only upon instrumental parameters and the mass and charge of an ion whereas the second term is very dependant upon the physico-chemical properties of an ion.) The fundamental rf amplitude determines the maximum kinetic energy of an ion in the ion trap which can be approximated by the Dehmelt pseudo-potential well (Eq. 2)

\[ D = q^2m_0\frac{\Omega^2}{2e} \]  

with the maximum kinetic energy then being

\[ mv^2 = 2eD \]  

Thus, for a given mass-to-charge ratio, the maximum kinetic energy of a trapped ion is proportional to \( q^2 \).

The effect of tickle duration is illustrated in Figure 1. At this \( q \) value (0.075) the maximum kinetic energy of the ions is 2.1 volts and they are ejected from the ion trap before they can be collisionally activated enough to dissociate. Note that 20 and 30 ms excitation periods show very little difference in behavior, which means that over the last 10 ms of a 30 ms excitation a steady state is reached i.e. the \( \frac{\text{d}v/\text{d}t}_{\text{collide}} \) term cancels the first term of the equation. At higher \( q \) values at which the ions can be sufficiently collisionally activated to dissociate there is a competition between ion dissociation and ion ejection. Dissociation is increasingly competitive at longer excitation times since more power can be absorbed by an ion at a given excitation voltage. Increasing the \( q \) value at a fixed excitation time again makes dissociation increasingly competitive with ion ejection due to the increasingly energetic collisions which can occur before ion energies sufficient for ejection are achieved.

Figure 2 shows the effect of changing the helium buffer gas pressure. At higher pressures more collisions occur within a given time period thus increasing the likelihood of CID versus ion injection. However since helium also affects the trapping efficiency its pressure affects the sensitivity. The top plot has a sensitivity of about a factor of 6 less than the middle plot and 3 less than the bottom plot.

Other collision gases can be added to the helium. Figure 3 shows plots of parent ion abundances ([M-H]^+ from N,N-dimethylamidine) and the sum of selected product ion abundances (solid symbols) with 0, 1 and 5 x 10^5 torr of Ar added to the helium. The trapping efficiency decreases as Ar is added to the system. At 1 x 10^5 there is not a lot of change from when no Ar is present which is due to the fact that only about 1 out of every 75 collisions of the parent ion is with Ar. However increasing this probability by a factor of 5 by going to 5 x 10^5 shows an earlier onset for dissociation and also the rate of dissociation increasing relative to the rate for ion ejection. When adding a heavier collision gas the main effect observed in the MS/MS spectrum for this ion is an increase in the relative abundance of the simple cleavage ions (m/z 105, 77, 51) versus the other, rearrangement ions. This is most dramatically demonstrated in Figure 4 which shows MS/MS spectra obtained with and without Xe added.
Coupling a Particle Beam and a Thermospray Interface to a Quadrupole Ion Trap Mass Spectrometer

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Quadrupole ion trap mass spectrometers have seen little use in LC/MS systems primarily because of the amount of solvent typically associated with LC introduction methods. Solvent ions cause space charging, reducing analyte sensitivity and mass range, while solvent neutrals may cause ion/molecule reactions resulting in undesired spectra. We have designed an ion trap instrument that provides additional means to reduce the unwanted matrix effects typically found at high solvent flow rates. The instrument uses differential pumping between the source and the ion trap analyzer and a DC quadrupole triplet lens is used to transmit the ions as shown in Figure 1. In addition to transmitting the ions to the trap, the DC quadrupole triplet serves as a beam-shaper and a region for improved pumping as shown in Figure 2. The instrument consists of Finnigan ITS40 electronics to control the mass selective instability scan function which is supplemented with axial modulation, the timing of the gate element, and the electron multiplier. Finnigan TSQ-70 electronics are used to control the source, vacuum pumps, ion gauge, and lens elements including two adjustable potentials for the gate lens. The element nearest the entrance endcap was used to gate the ions. The ion trap is floated to negative potentials when injecting positive ions from the grounded ion source and thus establishes the maximum initial collision energy inside the ion trap chamber. Helium, added directly to the trap, is necessary to trap injected ions [1] and gives a pressure of 2 x 10^- torr in the cradle. The source region is pumped with a 330 L/s turbomolecular pump while the analyzer region is pumped with 170 L/s and 330 L/s turbo molecular pumps.

The FC43 ion m/z=219 has been thought to go under fragmentation during injection when the ion source is located in close proximity to the ion trap. In the well pumped injection lens system described here, the relative abundance of m/z 219 is clearly observed as seen in Figure 3. A graph of ion intensity versus ion trap offset for the ions m/z = 69, 131, 219, and 502 from FC43 is shown in Figure 4. While m/z=69 is observed at ion trap offsets to at least -10 volts, m/z=219 is observed out to only -20 volts offset. All ions show a maximum intensity at approximately -6.5 volts. The shapes of the curves may be partially attributed to the Rf-amplitude during injection, ion optics, SID, and CAD in the trap or injection lenses.

To demonstrate the capability of LC/MS on the instrument, particle beam (PB) and thermospray (TSP) interfaces were coupled to the system. Figures 5-7 show the mass spectra from PB external EI ionization of caffeine, 17α-hydroxy-progesterone, and reserpine. The Rf-amplitude on the ring electrode was set to exclude ions less than 34 amu. Unlike a PB interface ion trap system using internal ionization [2], no self-CI is evident.

It is remarkable that the improved differential pumping also allows for the acquisition of resolved ions from a TSP interface. Adenosine and reserpine were both injected into a 1-1.5 ml/min flow of 0.1 M ammonium acetate made up in 30% methanol and 70% water. Figure 7 shows the typical TSP spectrum for adenosine which is believed to have gone under some thermal degradation of (M+H)^=268. The TSP spectrum of reserpine, (M+H)^+=609, is shown in Figure 8. Unit mass resolution is observed for all ions.


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Figure 1
DC QUADRUPOLE TRIPLET

Figure 2
ION INTENSITY vs ION TRAP OFFSET

Figure 3
ION INTENSITY vs ION TRAP OFFSET (VOLTS)

Figure 4

Figure 5
PB / EXTERNAL EI

Figure 6
PB / EXTERNAL EI

Figure 7
PB / EXTERNAL EI

Figure 8
THERMOSPRAY

(M + H)^+

Figure 9
THERMOSPRAY

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When operating the ion trap in the mass selective instability with resonance ejection mode, the resolution is dependent on the interaction time of the ion with the applied auxiliary field before the ion is ejected. Thus, given the appropriate choice of auxiliary field frequency and amplitude, the resolution can be increased by decreasing the scan speed of the Rf amplitude which brings ions successively towards resonance and causes ion ejection. The details and effects of the important parameters involved in obtaining high resolution is discussed in the following two abstracts. We describe here some experimental results demonstrating high resolution and its practical applications.

The instrument used for these studies is a highly modified ion trap mass spectrometer (ITMS) designed for the study of the biochemical applications of the ion trap. External ionization and subsequent axial ion injection is utilized. The available ion sources include EI, CI, Cesium ion desorption, laser desorption, and electrospray ionization. Our results demonstrating the first high resolution data obtained on the ion trap mass spectrometer operated in the mass selective instability mode with resonance ejection has recently appeared in the literature. Therefore, we discuss here the results of our study of the applications of high resolution for the combination of electrospray ionization with the ion trap mass spectrometer. Figure 1 shows the instrument configuration for the electrospray ion trap combination. It includes a Fenn-Whitehouse electrospray ion source, three stages of differential pumping, ion injection optics, and a 20 kV conversion dynode detector system.

Figure 2 shows a spectrum of horse skeletal myoglobin which includes an impurity or adduct ion peak associated with each peak of the multiply charged myoglobin. Such inhomogeneities are commonly found for biochemical systems and hinders the ability for accurate mass determination of proteins. Higher resolution allows the separation of the impurity peak from the pseudomolecular ion and therefore allows much more accurate assignment of protein molecular weight as well as aid in the identification of impurity or adduct ion species.

A practical application of high resolution is the separation of isotopes of multiply charged ions for the purpose of identification of the charge state. Figure 3 demonstrates the resolution obtainable for separating the isotopes of multiply charged ions using the doubly charged ion of gramicidin S. Figure 4 demonstrates that charge state determination can be performed using this method on parent, daughter and granddaughter ions using rennin substrate as the example.

In conclusion, high resolution can be achieved on an ion trap mass spectrometer operated in the mass selective instability with resonance ejection mode. A practical application of this resolution is the determination of charge states of electrosprayed ions, including multigeneration daughter ions. Limitations in the present method of achieving high resolution include instrument stability, space charge (especially for multiply charged ions), signal-to-noise loss due to detector bandwidth mismatch, and the use of high resolution for accurate mass measurement.

Figure 2.

Figure 3.

Figure 4.
Axial Modulation in the Paul Ion Trap: A Closer Look at Enhanced Resolution and Ion Motion During Resonance Ejection

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Although the mass-selective instability mode of operation of the quadrupole ion trap was very successful, a newer method of operation, the "mass-selective instability mode of operation with resonance ejection" (described in U.S. Patent No. 4,736,101) has proven to have certain advantages such as the ability to record mass spectra containing a greater range in abundances of the trapped ions. In this method of operation, a supplementary field is applied across the end cap electrodes and the magnitude of the r.f. field is scanned to bring ions of successively increasing m/z into resonance with the supplementary field so that they are ejected and detected to provide a mass spectrum. Commercially-produced ion trap mass spectrometers based on this mode of operation have recently become available, and these instruments have been successfully applied to an even wider variety of problems in chemical analysis than their predecessors.

Recently Schwartz and coworkers discovered that the newer scan method makes possible the acquisition of spectra with relatively high mass resolutions. Essentially, the scan rate (the volts per second ramp rate of the r.f. voltage supplied to the ring electrode) is reduced and an optimum resonance voltage is supplied. The enhancement of resolution with decreased scan speeds is in marked contrast to the behavior of the earlier mass selective instability mode. For example, Figure 1 shows spectra of xenon acquired at scan rates of 4.0E3 volts/second (3.1E2 amu/s), 6.4E5 volts/second (5E3 amu/s), and 1.02 volts/second (8E4 amu/s). At the high scan speed the resolution is somewhat degraded, at the intermediate scan rate the resolution is essentially unit resolution, and at the slow scan rate the resolution is not improved but is in fact somewhat poorer than at the usual scan speed of 5000 amu/second. Figures 2-4 show a comparison of the mass selective instability mode with and without resonance ejection for the same three scan rates. With resonance ejection, the resolution shows a strong dependence on scan rate and at the slowest scan speed, a resolution of much better than unit resolution is achieved.

The relatively weak dependence of the resolution on scan speed in the mass-selective instability mode makes it relatively simple to choose the optimum conditions for mass analysis: the trap is scanned at the maximum rate that does not lead to a loss of resolution (or that is not too fast for the data acquisition system). However, the strong dependence of the resolution on scan speed in the resonance-ejection scan forces one to optimize three parameters: the scan speed, the working point (i.e. the frequency of the resonance ejection voltage), and the amplitude of the ejection voltage. To seek the optimum scan conditions, we recently investigated the peak width (in time) of the m/z 129 peak of xenon at beta values between .56 and .95. A particular scan rate and working point were chosen and spectra were acquired over a large range of ejection voltages as shown in Figure 5. An optimum ejection voltage and associated peak width and resolution were obtained from each plot. The best resolution for each pair of conditions (scan rate and working point) is shown in Figure 6 and the corresponding peak width (in seconds) is shown in Figure 7.

The data generally indicate that the resolution decreases as the beta value becomes close to one and that a maximum resolution is reached in the vicinity of a beta of 0.73. The relatively weak trapping field near the edge of the stability diagram may account for the loss of resolution. Perhaps scattering causes a dephasing of the coherent ion motion which causes peak broadening; such an effect would become increasingly important as the edge is approached, as is observed. The decrease in resolution at lower beta values is probably due to the effective increase in scan rate as expressed in terms of amu per period of the ejection voltage. The combination of the two effects would lead to the observed maximum.

The linearity of plots of the logarithm of peak width vs the logarithm of scan speed (Figure 8) indicates a power function relationship. In this case, at very slow scan speeds the plot is parallel to the plot of the peak separation (in time), indicating that the resolution is not improved as the speed is decreased. We believe that space charge becomes increasingly important at the lower scan speeds and can account for the lack of improvement in resolution as the scan speed is decreased.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Xenon, Without Resonance Ejection

[Graph of mass spectra]

Figure 1

Xenon, 16X Speed (1.0E6 v/e)

[Graph of mass spectra with ejection voltages]

Figure 2

Xenon, *Normal* Scan Speed (6.4E4 v/e)

[Graph of mass spectra with ejection voltages]

Figure 3

Xenon, 1/16 Scan Speed (4.0E3 v/e)

[Graph of mass spectra with ejection voltages]

Figure 4

Resolution as a Function of Ejection Voltage

[Graph showing resolution vs ejection voltage]

Figure 5

Maximum Resolution vs Frequency and Scan Rate

[Graph showing maximum resolution vs frequency and scan rate]

Figure 6

Minimum Peak Width vs Frequency and Scan Rate

[Graph showing minimum peak width vs frequency and scan rate]

Figure 7

Peak Width for m/z 129

[Graph showing peak width for m/z 129]

Figure 8

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Considerations in the Choice of the RF Trapping Field Frequency for Ion Trap Mass Spectrometers Intended for High Mass (>5,000 Da.) Applications.

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To date, the best experimental results from attempts at extension of the mass range of the quadrupole ion trap instruments have been accomplished by the incorporation of resonant ejection into the mass selective instability scanning technique. In this experiment, resonant ejection of trapped ions at a $q_z$ well below the stability limit for the RF-only quadrupole ion trap, $q_z = 0.908$, allows a higher maximum mass to be brought to instability (resonance) and ejected upon application of the maximum available RF voltage. The primary factors in determining the mass range of the analysis are the frequency of the resonating supplementary field, and the maximum attainable intensity of the trapping field. The effective field intensity is determined by the trap size and the magnitude and frequency of the applied RF voltage.

Reported ion trap mass range extension experiments yielding mass ranges above ca. 5,000 Daltons have utilized modified commercially available ion traps having trapping field frequencies, $f$, of ca. 1 Mhz, trap characteristic dimension, $r_0$ of ca. 10 mm, and RF voltage amplitude, $V_s$ of about 7,000 Volts-peak at maximum. The supplementary field frequency, $f_v$, for these experiments are below 50 kHz. The amplitude of the supplementary voltage, $V_s$, is generally on the order of 1 Volt-peak. The an important issue is whether the instrument parameters are optimal for such high mass analysis. A review of the basic equations of motion of the ion trap yields some insight into this question.

The equation of motion of ions in the z dimension can be written as a forced Mathieu equation as shown in Equation (1).

$$\frac{d^2z}{dt^2} + 2q_z \cos(2\xi)z = p_z \cos(\alpha \xi)$$

The various normalized parameters in this equation are defined below in Equations (2),(3),(4), and (5).

$$\xi = \pi ft$$  \hspace{2cm} (2)  
\[ \alpha = \frac{f_v}{f} \quad \hspace{2cm} (3) \]

\[ q_z = \frac{eV}{m \beta_0 r_0^2 f_v^2} \quad \hspace{2cm} (4) \]
\[ p_z = \frac{eV_s}{\sqrt{2} m r_0^2 f_v^2} \quad \hspace{2cm} (5) \]

When considering the case where the mass range of an ion trap is being greatly extended by resonant ejection of ions at low $q_z$, this equation of motion can be well approximated by Equation (6). This simplification occurs because at low $q_z$, unforced ion motion is very nearly harmonic. The ion oscillation frequency in normalized units is $\beta_z$, which is approximately equal to $q_z/\sqrt{2}$ at low $q_z$. Resonant ejection and detection of ions occurs when the natural frequency of motion of a particular $m/e$, $f_n$, approaches the frequency of the supplementary field, $f_v$.

$$\frac{d^2z}{dt^2} + \beta_z^2 z = p_z \cos(\alpha \xi) \quad \text{where} \quad q_z < 4 \quad \text{and} \quad \beta_z = \frac{q_z}{\sqrt{2}}$$

While considering ion motion in such normalized terms is useful for providing basic understanding, further information appropriate to the choice of instrument operating parameters can be obtained by converting the normalized approximate equation of motion back to an unnormalized form as is given in Equation (7). Inspection of Equation (7) indicates that any combination of instrument parameters that yield same coefficient of $z$ should produce identical ion motion and therefore identical ion resonant frequencies. The ion resonant frequency, $f_{ion}$, is given in Equation (8).

During mass analysis, the trapped ions are successively ejected from the trap by linearly
increasing the amplitude of the trapping voltage. This means that, in practice, the coefficient of $z$ in Equation (7) is time variant. However any choice of trapping field frequency, $f$, and trapping voltage rate of change, $\frac{dV}{dt}$, which results an identical equation of motion for ions approaching resonance with a chosen supplementary field should result in identical ion trajectories during ejection. This condition for similarity will be satisfied any choice of $f$ and $\frac{dV}{dt}$ that provide the same mass scan rate, $\frac{dm}{dt}$. Therefore when resonant ejection is induced at low $q$, it is necessary to produce large mass range extensions, the choice of mass scan rate, $\frac{dm}{dt}$, and the applied supplementary field frequency, $f_s$, will determine the achieved mass resolution. Mass resolution should be independent of the trapping field frequency, $f$.

However the mass range of the instrument will depend on the choice of the trapping field frequency. Equation (9) shows how mass range depends on the relevant instrument parameters.

\[
\text{Mass range, } m_{\text{max}}, \text{ is shown to be proportional to the maximum available trapping voltage } V_{\text{max}} \text{ and inversely proportional to both the supplementary field frequency, } f_s, \text{ and the trapping field frequency, } f. \text{ This means that the mass range of the instruments can be extended without loss in mass resolution if the trapping field frequency is reduced while maintaining the same supplementary field frequency and maximum trapping field voltage. Alternatively, this also means that a higher supplementary field frequency can be used obtain a desired mass range for a given maximum trapping field voltage if a proportionally lower trapping field frequency is also used.}

There is advantage in maximizing the supplementary field frequency used to obtain a given mass range. This is because the dispersion in ion resonant frequency with mass, $df/\frac{dm}{dm}$, for ions close to resonance with the supplementary field will strongly affect the mass resolution obtained for a given mass scan rate. The mass selective instability scan with resonance ejection experiment essentially measures the resonant frequency of stored ions. The mass scan rate effectively controls the observation time and the resonant frequency dispersion specifies the necessary frequency resolution required to distinguish adjacent masses. The slower the mass scan rate the higher the resolution. The greater the frequency dispersion the better the mass resolution. Equation (10) is an expression for ion resonant frequency dispersion, $df/\frac{dm}{dm}$, in terms of supplementary field frequency, $f_s$, and the ion mass at resonance, $m_{\text{eject}}$.

\[
\frac{df}{dm} = \frac{f_s}{m_{\text{eject}}}
\]

This expression clearly shows that ion frequency dispersion for a given mass scan rate increases proportionally with the supplementary field frequency. Thus attainable mass resolution for a given mass scan rate should increase with increased supplementary field frequency.

These characteristics were tested experimentally by operating a standard 10 mm $r_0$ ion trap at three different trapping field frequencies: 1100 kHz, 762.7 kHz and 550 kHz. The mass resolution obtained at a mass scan rate of ca. 135 da/sec with a supplementary field frequency of 35.371 kHz was studied by observing the m/z 502 and m/z 503 from FC-43. The observed resolutions were nearly equal for all three trapping frequencies. When the supplementary field frequency was increased improved resolution at the same mass scan rate was observed. Also the ability to increase mass scan rate at a given resolution by using higher supplementary field frequencies was also observed.
Initial Interpretation of High Energy Collisionally Induced Decomposition Spectra obtained from a Known and Unknown Peptide in the Mass Range 60-2,000 Daultons by MS/MS.

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Interpretation of collisionally induced decomposition spectra (daughter ions) of an unknown peptide from a tandem mass spectrometer or by a constant B/E link scan on a two sector magnetic instrument is not a trivial matter.

The initial step is to identify all potential amino acid residues and their position in the sequence by generating a list of ion peak pairs from the spectrum whose mass difference corresponds to an amino acid residue. Listing them in order of increasing mass, based on the low mass ion peak in each pair.

From this list (a) create a series of branched tree structures, built up from the ion pairs, which represents all the "potential" amino acid sequences possible from the experimental data present in the spectrum, Fig 1 shows one of the branched tree structures obtained from the spectrum of Gramicidin-D. (b) Scan through the sequences, one at a time, picking out the amino acid residue, and if the sequence contains six or more amino acids residues, an arbitrary number, calculate the N and C terminal fragments. As the program passes through the list of ion pairs a flag is raised at each pair to indicate which pairs have been used. These flags prevent the program using these pathways or portions of pathways again.

The sequences from (b) above are examined for relationships which may indicate a potential or partial sequence for the unknown peptide in question. For example, the two potential sequences obtained from the mass spectrum of Gramicidin-D are shown in Fig 2, the second sequence is the inversion of part of the first sequence. After inverting the second sequence and rewriting them again side by side one can create a third sequence which corresponds to the sequence present in Gramicidin-D, except one Valine (VAL) is missing from the low mass end of the peptide.

It must be pointed out that that this process cannot distinguish between
It must be pointed out that this process cannot distinguish between the N and C terminal ends of the peptide. In (b) above the software assumes the low mass end of the amino acid sequence is the N terminal.

Acknowledgments. We wish to thank Fred Walsh of the University of California, San Francisco and Kratos Analytical for running the Gramicidin-D spectra.

**Fig 1.**

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**Fig 2.**

**Sequence 1**

GLU-VAL-ALA-VAL-VAL-VAL-TRP-LEU-TRP-LEU-TRP-LEU-TRP-

**Sequence 2**

LEU-TRP-LEU-TRP-VAL-VAL-ALA-LEU-ALA-GLY-

GLU-VAL-ALA-VAL-VAL-VAL-TRP-LEU-TRP-LEU-TRP-LEU-TRP-GLY-ALA-LEU-ALA-VAL-VAL-VAL-TRP-LEU-TRP-LEU-

**Sequence 3**

GLY-ALA-LEU-ALA-VAL-VAL-VAL-TRP-LEU-TRP-LEU-TRP-LEU-TRP-
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MASS SPECTROMETRIC MEASUREMENT OF \( \beta \)-ENDORPHIN AND METHIONINE ENKEPHALIN IN HUMAN PITUITARIES

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Two opioid neuropeptides, \( \beta \)-endorphin (BE) and methionine enkephalin (ME), were quantified with fast atom bombardment mass spectrometry (FAB-MS) in individual human pituitaries (post-mortem) and in tumor pituitaries (post-surgery) in a study to clarify the molecular processes that occur in tumor formation. FAB-MS in the multiple reaction monitoring (MRM) mode was used to link the MH\(^+\) precursor ion of the peptide with a fragment ion that was unique to each neuropeptide and to increase significantly the molecular specificity of these quantitative analytical measurements. The experimental procedure follows our general scheme 1,2,3. Briefly, the human pituitaries obtained at autopsy were frozen immediately and kept at -70°C until analysis. Frozen tissue was weighed, placed immediately into cold (4°C) acetic acid (1 N, 1:20 w/v), and homogenized. After 2 h, the homogenate was centrifuged (31000g; 30 min), and the supernatant was prepurified with Sep-Pak. The peptide-rich fraction was lyophilized, reconstituted into 150 \( \mu \)l of mobile phase, and subjected to gradient RP-HPLC separation. The ME fraction was lyophilized, reconstituted into 150 \( \mu \)l of mobile phase and analyzed on polymer column. The collected ME fraction was lyophilized, and analyzed by mass spectrometry. The gradient HPLC BE fraction from the pluillitary extracts and the standard curve solutions were subjected to tryptic digestion. The tryptic digest was purified with Sep-Pak and analyzed on ODS HPLC column. The isocratic fraction containing NAIIIK was lyophilized; and reconstituted in 25 \( \mu \)l of 0.1% TFA for MS analysis. Two corresponding stable isotope-incorporated peptides, 10 \( \mu \)g of [\(^2\)H\(_5\)-Phe]-ME and 10 \( \mu \)g of [\(^2\)H\(_4\)-2\( \alpha \)-Ile]-BE1-31, human 4, respectively, were added before tissue homogenization as internal standards. ME was quantified as the intact pentapeptide, and BE1-31 was quantified via its tryptic fragment BE20-24 (NAIIIK). The amount of each neuropeptide quantified in control post-mortem pituitaries (n=8) was 75.2 ± 29.6 (s.e.m.) pmol ME \( \mu \)g\(^{-1}\) protein and 132.5 ± 22.3 (s.e.m.) pmol BE \( \mu \)g\(^{-1}\) protein and in the pituitary tumor samples (n=5), 25.0 ± 7.6 pmol ME \( \mu \)g\(^{-1}\) protein and 36.0 ± 14.6 pmol BE \( \mu \)g\(^{-1}\) protein 3. The difference in the BE content between the control and tumor pituitaries was significant (p=0.004) (Fig. 1), and may reflect an aberrant metabolism of the POMC system in these human pituitary tumor tissues. The measurements from FAB-MS methods (MH\(^+\) ion monitoring and the MRM mode) were compared to measurements from radioreceptor assay and RIA by analyzing the amount of endogenous ME extracted from five human post-mortem pituitary samples. ME-like reactivity (ME-LR) was 116 ± 27 pmol \( \mu \)g\(^{-1}\) protein (s.e.m.); ME-like immunoreactivity (ME-LI) was 18.3 ± 9.5 pmol \( \mu \)g\(^{-1}\) protein; MH\(^+\) data were 42.9 ± 9.5 pmol ME \( \mu \)g\(^{-1}\) protein; and the MRM data were 47.7 ± 12.7 pmol ME \( \mu \)g\(^{-1}\) protein. The highest level of molecular specificity amongst these four analytical methods to measure picomole amounts of endogenous ME was the FAB MS-MRM method (Fig. 2) 2.

References:

Supported by NIH GM 26666.
BE AND ME IN HUMAN PITUITARY

Fig. 1. Histogram of the MRM measurements of BE and ME in controls (left bars) and tumors (right bars).\(^3\)

ANALYTICAL METHOD

Fig. 2. Plot of the average amount of native ME measured in a pituitary by the four analytical methods RRA, RIA, FAB-MS using MH\(^+\) and FAB-MS using SRM.\(^2\)
INTRAMOLECULAR $^{18}$O ISOTOPIC EXCHANGE OCCURRING IN THE GAS PHASE OBSERVED DURING THE HYBRID TANDEM MASS SPECTROMETRIC ANALYSIS OF PEPTIDES. Kevin D. Ballard and Simon J. Gaskell, Center for Experimental Therapeutics, Baylor College of Medicine, Houston TX 77030.

A fragmentation process involving a rearrangement mechanism occurring in fast atom bombardment desorbed protonated and metal cationized peptide ions during tandem mass spectrometric analysis has been previously studied by others\(^1\) and in our laboratory\(^2\). During this rearrangement, loss of the C-terminal amino acid residue is accompanied by transfer of an OH group from the C-terminal carboxylic acid moiety to the carbonyl group of the final peptide bond, giving rise to a product ion which may be formally designated as $[B_{n-1} + \text{OH}]^{+}$ or $[B_{n-1} + \text{Cat} + \text{OH}]^{+}$. This ion is mass spectrometrically indistinguishable from the $[M+\text{H}]^{+}$ ion of the corresponding peptide shortened by one amino acid residue at the C-terminus\(^2\). The transfer of carboxyl oxygen from the original C-terminus to the new C-terminus was demonstrated using $[^{18}$O$]_{2}$-labelled peptides, with both $^{18}$O atoms located in the C-terminal carboxylic acid group\(^2\). Product ion spectra of these compounds revealed rearrangement species which retained one of the $^{18}$O-labels ($[B_{n-1} + ^{18}$OH$]^{+}$). Also observed were less intense signals corresponding to rearrangement species which retained both of the $^{18}$O-labels. The mechanisms which have previously been proposed for the rearrangement process for both metal cationized\(^3\) and protonated\(^4\) peptides do not account for the retention of both $^{18}$O atoms in the rearrangement products. The present studies were undertaken to address this issue using a variety of hybrid tandem MS (BEqQ) approaches.

RESULTS Many of the peptides which undergo this rearrangement process also undergo a second stage of rearrangement, i.e. the $[B_{n-1} + \text{OH}]^{+}$ ions further rearrange to yield $[B_{n-2} + \text{OH}]^{+}$ ions. With $[^{18}$O$]_{2}$-labelled peptides, the $[^{18}$O$]_{2}$-labelled $[B_{n+1} + \text{OH}]^{+}$ ions would be expected to give rise to both unlabelled and $[^{18}$O$]_{2}$-labelled $[B_{n+2} + \text{OH}]^{+}$ ions in a ratio of 1:1. For the $[^{18}$O$]_{2}$-labelled $[B_{n-1} + \text{OH}]^{+}$ ions, if both labels are located at the C-terminus, then these ions would be expected to yield $[^{18}$O$]_{2}$-labelled $[B_{n+2} + \text{OH}]^{+}$ ions but no unlabelled $[B_{n+2} + \text{OH}]^{+}$ ions. If the second label is located somewhere other than at the C-terminus, then both $[^{18}$O$]_{2}$- and $[^{18}$O$]_{2}$-labelled $[B_{n+2} + \text{OH}]^{+}$ ions would be expected in a 1:1 ratio. In order to examine directly the sequential rearrangement processes, $[^{18}$O$]_{2}$- and $[^{18}$O$]_{2}$-labelled first generation rearrangement ions were analyzed by second generation product ion scanning using the sequential MS capability of a BEqQ hybrid mass spectrometer\(^5\). Figure 1(a) shows a portion of the first generation product ion spectrum of sodium cationized $[^{18}$O$]_{2}$-labelled fragments obtained under metastable detection conditions. Figure 1(b) presents the second generation product ion scan obtained from the $[^{18}$O$]_{2}$-labelled rearrangement ion. The unlabelled and $[^{18}$O$]_{2}$-labelled second generation rearrangement ions were detected in roughly equal abundance. The corresponding scan obtained from the $[^{18}$O$]_{2}$-labelled first generation rearrangement ion is shown in Figure 1(c). The major signal corresponded to the $[^{18}$O$]_{2}$-labelled second generation rearrangement ion, although a small but significant (and reproducible) signal corresponding to the $[^{18}$O$]_{2}$-labelled second generation rearrangement ion was also detected. Similar results were obtained with protonated TRKR. These data strongly suggest that the two $^{18}$O atoms in the $[^{18}$O$]_{2}$-labelled first generation rearrangement ions were located in the C-terminal carboxyl group, so that both of these atoms were transferred from the original C-terminus to the C-terminus of the first generation rearrangement ion. The distortion from a 1:1 ratio in Figure 1(b), as well as the presence of detectable amounts of $[^{18}$O$]_{2}$-labelled second generation rearrangement species (Figure 1(c)), suggest that both of the carboxyl oxygens can be transferred to the new C-terminus even through two generations. A mechanism which would explain the formation of the $[^{18}$O$]_{2}$-labelled rearrangement ions would involve steps prior to bond cleavage which are reversible, enabling intra-ionic isotope exchange to occur without bond cleavage (Scheme I). The isotopically exchanged species could then undergo the rearrangement to generate the $[^{18}$O$]_{2}$-labelled rearrangement ion. A similar mechanism can be envisaged for the metal cationized species. Conventional product ion spectra revealed the presence of both $[^{18}$O$]$- and $[^{18}$O$]$-labelled Y\(_{n}$) ions from several $[^{18}$O$]$-peptides, indicating $[^{18}$O$]$ depletion from the C-terminus in a portion of the precursor ion population. Such spectra also revealed fragment ions which show isotope enrichments consistent with the incorporation of $^{18}$O at the peptide bond between the penultimate and C-terminal amino acid residues. Several lines of evidence eliminated the possibility of such incorporation of label during solution phase preparation of the $[^{18}$O$]$-labelled analogues. Thus, the combined data are in accord with an isotope exchange process occurring in the gas phase, most likely through a mechanism involving the reversible formation of a cyclic intermediate. These studies, combined with previous data, emphasize the importance of the conformations adopted by gas phase peptide ions in influencing fragmentation pathways.

ACKNOWLEDGEMENT. This work was supported in part through a generous gift from Glaxo, Inc.

Sodium Cationized YGGFL

Figure 1.

Scheme I.
RAPID CONFIRMATION AND REVISION OF THE PRIMARY STRUCTURE OF BOVINE SERUM ALBUMIN BY ESIMS AND FRIT-FAB LC/MS

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Central Research Laboratories, Ajinomoto Co. Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki, Kanagawa 210, Japan

INTRODUCTION
Since the base sequencing technique of DNA was established, an opportunity for the determination of the full sequence of a protein only by the conventional methods has been more and more decreasing. Instead of the conventional sequencing techniques of a protein, a method for the rapid confirmation of the amino acid sequence of a protein, which was presumed by the base sequencing technique, is coming up to be required. From such a viewpoint, we studied the primary structure of bovine serum albumin (BSA) by electrospray ionization mass spectrometry (ESIMS) and frit-fast atom bombardment mass spectrometry/liquid chromatography (Frit-FAB LC/MS), and revised its sequence by tandem mass spectrometry and automated Edman degradation.

RESULTS and DISCUSSION
Figure 1 shows an electrospray ionization mass spectrum of BSA and its deconvoluted spectrum. The molecular weight of BSA was determined as 66436.0 by ESIMS, which was obviously larger than that of the theoretical value calculated from the primary structure of 582 amino acid residues (average molecular weight: 66267.1). In consideration of the accuracy of ESIMS for other proteins (1), the difference of these two values was too large to interpret it as a measurement error. Therefore we thought that the amino acid sequence of BSA composed of 582 residues must be incorrect. So we tried to reinvestigate the amino acid sequence of BSA by mainly using Frit-FAB LC/MS with the combination of automated Edman degradation and tandem MS. Figure 2 shows the revised amino acid sequence of BSA (2). The previous sequence was composed of 582 amino acid residues determined by Edman degradation method (3-5). In the previous sequence a tyrosine residue at the 156th was lacked, and the sequence at the positions of 94th and 95th, that was -EQ-, was revised to -QE-. The methodology is as follows: First, reduced and carboxymethylated BSA (RCM-BSA) is digested by trypsin. Second, the molecular weights of all the peptides are determined by Frit-FAB LC/MS. These values are compared with the molecular weights of the tryptic peptides predicted from the amino acid sequence of BSA. These peptides are matched to the sequence on the basis of their molecular weights and specificity of the enzyme. Third, if there is ambiguities presented by the possibility of having more than one peptide with the same molecular weight, immonium ions in the Frit-FAB mass spectrum (6) or by daughter ions from the protonated molecular ion of the peptide (7-9) is used for the decision of the sequence. Finally, the peptide, which cannot be assigned to the sequence by its molecular weight and the specificity of the enzyme, is isolated by HPLC and sequenced by Edman degradation method and tandem MS.

Seventy-five peaks were observed in a reversed phase HPLC chromatogram of tryptic peptides of RCM-BSA (Fig. 3). Identification of 66 peptides in tryptic digests confirmed 93% of the sequence of the protein. There were two peptides whose molecular weight could be matched to more than one regions in the sequence, considering enzyme specificity. The positions in the sequence of these two peptides were deduced by taking account of immonium ions in the Frit-FAB mass spectrum or by daughter ions from the protonated molecular ion. And one peptide could be matched to no position in the sequence. Unidentified last peptide in the peak 66, whose molecular weight was 1177.3, was isolated and sequenced by automated Edman degradation to FYAPELLEY. In addition to that, the sequence of -LYY was clearly confirmed by tandem mass spectrometry. The calculated molecular weight from the sequence of this peptide (MW:1177.6) agreed well with the observed value. In comparison with the sequence in this region of human serum albumin (FYAPELLEFF) and rat serum albumin (FYAPELLEY), revised sequence of FYAPELLEY is reasonable and a lack of 156th tyrosine is obvious. It took about two weeks to confirm 93% of the sequence of this protein. In order to confirm remaining 7% of the sequence, RCM-BSA was digested by lysyl endopeptidase or Staphylococcus au-
V8 protease. After the digestion by these enzymes, peptide mixture was subjected to Frit-FAB LC/MS and molecular weight of each peptide was determined. In the end, comparing with the other two serum albums, the order of glutamic acid and glutamine residues at the positions of 94th and 95th of BSA was doubtful. The sequence of these positions was corrected to -QE- by automated Edman degradation of a lysyl endopeptidase digested peptide of QEPERNECFLSHK (Gln94 to Lys106).

ACKNOWLEDGEMENT

We thank Finnigan MAT Instruments, Inc., for operating electrospray ionization mass spectrometer.

REFERENCES

AMP Nucleosidase (AMN) catalyzes the hydrolysis of the N-glycosidic bond of adenosine monophosphate (AMP), into ribose-5-phosphate and adenine.

A deletion construct which eliminates amino acids 128-135, was made in the E. coli protein. The structure of the deletion mutation was characterized by HPLC-FABMS analysis of enzymatic digests of both the native and mutant proteins. Combined trypsin (cleavage at Lys, Arg) and V-8 protease (Glu) digests of each protein were prepared.

LCMS experiments were performed with an ABI 130 syringe-pump HPLC and a Finnegan-MAT 90 (BE geometry) equipped with a continuous-flow interface and modified to accept a stainless steel frit. Xenon FAB desorption / ionization was accomplished with an Ion-Tech 8 Kev saddle-field gun. Samples were separated by a reversed-phase, microbore HPLC column (Aquapore RP-300, C-8, 1.0 x 100 mm) and introduced directly into the source without flow splitting at 10 μl/min, using a gradient of 3%->50% (v/v) acetonitrile in 5% glycerol / 0.1% trifluoroacetic acid. We have now determined that use of the frit allows lower concentrations of glycerol to be used in the HPLC solvents. The use of lower glycerol concentrations also allows higher acetonitrile concentrations to be used for the elution of the peptides without precipitation of the glycerol out of solution. Accumulated scans (mass summary plots) were used to assign most of the ions to the predicted masses of the peptides (Table 1). Those peptides forming weak ions were located by using selected ion chromatograms. Of the 40 peptides predicted for AMN within the mass range 450-2200 amu, 34 peptides were confirmed (Table 1). Some peptides were identified as products of incomplete digestion. The deletion sequence (boxed in Fig. 1), contains a single, V-8 cleavage site which produced two unique peptides in the wild-type protein digest (VT-16 and VT-17 Fig 2), while the deletion mutant produced a single unique peptide (VT16m) with sequences flanking the deletion site. Selected ion chromatograms (Fig 3) show the elution positions of these peptides, along with that of a peptide fragment common to both digests (VT-66, m/z=710). Spectra of the peptides are presented in Fig. 4. Static FAB analysis of the digest mixture was unable to identify the critical sequences. These experiments demonstrate the utility of LCMS in protein analysis.
Figure 3. Selected Ion chromatograms for peptides unique to wild and mutant proteins.

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Figure 4. FAB mass spectra (5KV) of VT 16, VT 17 and VT 15m.

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**Figure 3:**
- VT17 LTLDR
- VTSC AZDIJJI
- VT1S UTPYVIDOfII

**Figure 4:**
- VT48 531 33
- VT15 | 851.0 | 358 - 360
- VT17 | 851.0 | 358 - 360
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**References:**
In the past few years, electrospray ionization (ESI) has emerged as the ultimate ionization technique for the on-line analysis of peptides and proteins. In our initial attempts to couple reverse phase high performance liquid chromatography (HPLC) to ESI it was found that there were many compatibility problems with the two techniques. For example, conventional electrospray is not compatible with 100% aqueous solutions due to the high liquid surface tension. Additionally, it was found that solutions of high conductivity (e.g., 0.1% trifluoroacetic acid, TFA) dramatically reduced ion current stability and sensitivity. Recent improvements to our interface have enhanced sensitivity and ion current stability for these types of mobile phases. A multilayer flow electrospray system (shown in Figure 1) delivers a sheath liquid flow and a concentric gas flow at the terminus of the ESI needle in order to allow for efficient droplet dispersion (and ion production) over a wide range of mobile phase conditions. A small mixing zone exists at the ESI tip, which allows the LC eluate to mix with a sheath liquid of relatively low surface tension. The focussing (or sheath) gas flow improves stability (by preventing droplet build-up on the tip of the needle) and directs more droplets at the capillary entrance. Typically a 1:1 eluate:sheath liquid flow ratio is utilized in order to maintain low surface tension over a gradient separation. For positive ion applications employing TFA, it was found that the use of 2-methoxyethanol as the sheath liquid provided the best sensitivity.

Additional sensitivity improvements have been made by incorporating an electrostatic tube lens in the viscous flow region between the capillary exit and the skimmer orifice, as shown in Figure 1. The tube lens increases the fraction of ions entering the ion optics and mass analyzer by forcing ions in the expanding free jet back toward the central axis.

When used with conventional-bore HPLC columns (1 mm - 4 mm i.d.), a post-column split is utilized so that the optimal flow range of 2-3 µL/min is delivered to the ESI interface. Since ESI behaves as a concentration-dependent detector, the use of high split ratios is not a disadvantage. In fact, for conventional bore columns, greater than 95% of the sample may be recovered via fraction collection for subsequent analysis. However, the use of small bore columns (e.g., 0.2 mm - 1 mm i.d.) requires the least amount of sample injected, since eluate concentrations are higher than what would be obtained if the same amount were to be injected on larger bore columns operating at higher flow rates. This is demonstrated in Figure 2, which shows a micro-LC/MS separation of a calmodulin tryptic digest on a 0.2 mm i.d. column with only 4 picomoles of sample injected. For micro-column applications, no post-column split is necessary, since the column flow rate is only 2 µL/min. However, a pre-column (pre-injector) split is necessary so that gradients are delivered to the column without long delay times. It is also possible to obtain sequence information at low picomole levels with this type of column as shown in Figure 3, which demonstrates the daughter spectrum of the doubly-charged tryptic fragment, Tg, of calmodulin.

Another example, demonstrating the value of on-line characterization of protein enzymatic digests is shown in Figures 4 and 5 for the analysis of human hemoglobin on a 2 mm i.d. column. The existence of a variant is readily identified by visual inspection of the two LC/MS chromatograms of Figure 4. The molecular weight of the variant was determined experimentally from this data to be 2043.4 Da. From the LC/MS/MS data of the triply-charged ion of the variant (Figure 5), sequence information was obtained that confirmed the variant contained a glutamic acid (E) residue instead of the normal lysine (K) at the 16 position on the α-chain.

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The authors would like to acknowledge H. Ewa Witkowska of Oakland Children's Hospital, who worked with us on the hemoglobin analysis.
Laser Desorption and Electrospray MS of the Scrapie Prion Protein.

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The scrapie prion protein (PrPSc) has been implicated in the development of a number of animal and human neurodegenerative diseases (1). Syrian hamster PrPSc has a phosphoinositol glycolipid (GPI) anchor at the C-terminus (2), and the majority of molecules are doubly glycosylated at Asn-181 and 197 (3). It is encoded by a single-copy gene that also encodes a normal cellular protein (PrPc) (4).

Detailed analysis of these proteins by enzyme digestion, HPLC separation and characterization of the resulting peptides by amino acid analysis, Edman sequencing and LSIMS, has been unsuccessful in revealing any differences between them (5). We have now used matrix-assisted laser desorption (LD) and electrospray (ES) to study the intact proteins and larger peptides and some mid-size peptides (2,000-3,000 Da) that were not readily amenable to LSIMS, presumably because of unfavorable surface activities. We have also obtained ES mass spectra of the C-terminal undecapeptide with the GPI anchor from an endoproteinase Lys-C digest. LD was carried out on a time of flight MS built at Rockefeller University and ES was on a VG Bio-Q.

Molecular mass of the intact protein. Purification of PrPSc from brains of hamsters with scrapie generally relies on the resistance of this protein to protease digestion. Proteinase K removes approximately 67 amino acids from the N-terminus of PrPSc to generate PrP 27-30 which contains two N-linked oligosaccharides and the GPI anchor. After reduction and alkylation of the cysteines with iodoacetic acid, the protein was dissolved in 70% formic acid containing sinapinic acid, deposited on the laser target, and dried. The LD spectrum (Figure 1) revealed three broad unresolved signals which we attributed to the heterogeneity of the oligosaccharides and the anchor glycan, the presence of some unglycosylated protein, and a ragged N-terminus due to multiple cleavage sites for proteinase-K. The ragged N-terminus was found by ES analysis of the N-terminal peptides from a Lys-C digest. The uncertainty introduced by this large degree of heterogeneity made it difficult to use the spectrum in Figure 1 to determine the molecular weight of the protein.

Using modified purification protocols without proteinase K, the full length protein was isolated, thereby eliminating the heterogeneity associated with the ragged N-terminus. After reduction and alkylation this was deglycosylated by the action of the enzyme PNGase F to eliminate the heterogeneity due to the N-linked oligosaccharides. The LD spectrum shown in Figure 2 gave a broad molecular ion peak (approx 1,000 Da) as the heterogeneous species due to the various anchor glycans were not resolved. The mass at the peak top (25,526 Da) measured in this experiment at Rockefeller University was 66 Da higher than what was obtained from a measurement made on a similar instrument (Vestec), reflecting the uncertainty of the experiment due to the inadequate resolving power. Nevertheless, this was within the band of anticipated molecular masses that we calculated to be 25,185 - 25,638 Da and it indicates that there probably are no other major posttranslational modifications. It is anticipated that ES with a quadrupole analyser would provide sufficient resolving power to separate the various molecular species. As yet we have not found a suitable solvent for this hydrophobic protein that is also compatible with ES.

A comparison of LD and ES analysis of a large glycopeptide. A 75-amino acid residue containing the first glycosylation site was obtained by reverse phase HPLC after digestion with Lys-C. The LD spectrum (Figure 3) was poorly resolved but showed sufficient structure to permit mass assignments for glycopeptides with 4-5 different oligosaccharides. ES gave very similar molecular masses for the major components (Figure 4) but the enhanced resolution revealed much more structure and it was possible to determine the molecular masses of at least 28 different glycopeptides in the range 10,256 - 11,388 Da. After deglycosylation with PNGase F, ESP gave the predicted mass of 8,608 Da for the peptide (data not shown), confirming that the heterogeneity was entirely due to the oligosaccharides.

ES analysis of the GPI anchor. The peptide mixture from a Lys-C digest was treated with the enzyme PIPLC to remove the lipid portion of the GPI. The C-terminal peptide - GPI anchor was separated by reversed phase HPLC and analysed by ES from 1:1 methanol/water matrix containing 1% acetic acid to give the spectrum shown in Figure 5. In separate experiments the masses and structures of the peptide (6) and glycan portions (7) were determined after treatment with 50% aqueous HF cleaved the phosphate bonds. Figure 5 showed three separate species which confirmed the heterogeneity of the glycan. Subsequent improvements to the methodology allowed the separation and ES analysis of individual components in the mixture with enhanced resolution. The spectrum also revealed the presence of two phosphoethanolamines, one more than the minimum of two required
for all GPI anchors, and consistent with the known structures of the acetylcholinesterase and Thy-1
GPI anchors. On the basis of this and other data (8) and by analogy with other known structures we
propose that species C in the ES spectrum is compatible with the structure shown in Figure 6.

Acknowledgements. This work was supported by NIH grants AG02132, AG08967, NS14069 and
NS22786 (S.B.P.) and RR01614 (A.L.B.)

References.

Figure 1. LD spectrum of PrP27-30

Figure 2. LD spectrum of PrPSc after PNGase F

Figure 3. LD spectrum of glycopeptide

Figure 4. ES spectrum of glycopeptide

Figure 5. ES spectrum of C-terminus-GPI

Figure 6. Structure of anchor species C
Inductively Coupled Plasma Mass Spectrometry as a Tool in Investigations of Trace Element Metabolism. Morteza Janghorbani, Department of Medicine, University of Chicago, Chicago, IL 60637.

Introduction. Inductively coupled plasma mass spectrometry is a versatile tool for isotopic analysis of a broad range of chemical elements in biological materials (1). It has been applied to metabolic studies of lithium (Li), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), and selenium (Se). These studies have included issues of gastrointestinal absorption and various dietary/host factors that modulate it, significance of different routes of excretion to the overall maintenance of homeostatic control, dynamics of distribution in various body compartments, and issues related to in vivo measurement of pool sizes.

Examples of Applications. In order to illustrate the wide range of potential applications of this approach to elucidation of various aspects of mineral metabolism, I present a brief discussion of two specific applications that we have recently discussed (2,3): measurement of exchangeable pool of magnesium (Mg) in infants (2), and applications of a continuous stable isotope feeding protocol to the measurement of plasma-organ fluxes of Cu in the adult rat (3).

1- Exchangeable Pool of Mg in Infants (2). Assessment of Mg status is a difficult problem in part because the major portion of soft-tissue Mg is intracellular and that changes in intracellular Mg content are not generally reflected in similar changes in plasma Mg. We carried out a feasibility experiment to establish whether the concept of in vivo isotope dilution could be applied to the measurement of exchangeable Mg in infants with the stable isotope approach. Three infants were given enriched \(^{25}\)Mg (dose range 2.4-7.1 mg/kg), here called "the label", in divided doses administered over a 24-h feeding period. Quantitative collections of stools and urine were made over the following 72 hours and a single sample of blood was obtained at 72 hours. Both the isotope ratio \(^{25}\)Mg/\(^{26}\)Mg and total Mg content of each sample were measured.

The data demonstrated that when a dose range of 2.4-7.1 mg \(^{25}\)Mg/kg is administered to the infants orally, plasma (and urine) isotopic enrichment of 6.3-28.4% is achieved at 72 hours. Since an isotopic enrichment of \(~10\%\) appears to be sufficient for accurate measurement of the isotope dilution space (1), this protocol provides a feasible approach to the measurement of exchangeable body Mg. Now that the feasibility of this measurement is established, the biological issues of the exchangeability of body Mg under various conditions of nutritional status, health and disease, and the role of skeletal Mg in relation to exchangeable body Mg need to be investigated.
2- Regulation of Copper Turnover in the Rat (3). Copper is an essential micronutrient. Organs are able to conserve their Cu when dietary sources become limited. However, little is known about the mechanisms for this conservation. This is in part due to inability to measure changes in components of organ Cu flux (plasma-organ influx/efflux) under steady-state conditions relevant to the whole animal. These experiments cannot be performed with the radioisotopes of Cu.

We have taken advantage of the fact that Cu naturally occurs as two stable isotopes ($^{63}$Cu, 68.4 wt%; $^{65}$Cu, 31.6%). Animals can be fed a Cu-free diet whose Cu content has been adjusted to the desired level with one of the two stable isotopes, say $^{63}$Cu. Measurement of the dynamics of changes in organ content of the two isotopes during this turnover phase will then provide an experimental approach for investigation of plasma-organ Cu fluxes under various experimental conditions.

Adult rats were fed a Cu-deficient diet (0.8 mg Cu/g) and provided with water containing 20 μg Cu/mL as $^{63}$Cu (99.7%) for 60 days. Three animals were killed on each of days 0 (prior to switching to $^{63}$Cu), 1, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56. Various organs and plasma were analyzed quantitatively for total Cu and the ratio $^{63}$Cu/$^{65}$Cu. From these measurements, the time course of organ (and plasma) endogenous Cu (Cu$_e$; Cu originating from body Cu at t=0) and exogenous Cu (Cu$_x$; total Cu - Cu$_e$) were established. Each set of data was then fitted to an appropriate mathematical expression describing the time dependence of Cu$_e$ and Cu$_x$ for each organ and plasma.

The two components of plasma-organ flux (influx/efflux) can be described mathematically by two simultaneous differential equations. Solution of these equations then provides the numeric values of rate constants from which rates of flux between plasma and the organ can be calculated.

Data given in Table 2 summarize the flux rates calculated as the average values for days 20-60 of the experiment.

Table 2- Flux rates between plasma and selected organs in adult rats

<table>
<thead>
<tr>
<th>Organ</th>
<th>Influx (μg Cu/d)</th>
<th>Efflux (μg Cu/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>4.15±0.42</td>
<td>3.85±0.49</td>
</tr>
<tr>
<td>Heart</td>
<td>0.097±0.0052</td>
<td>0.095±0.0062</td>
</tr>
<tr>
<td>Brain</td>
<td>0.021±0.0009</td>
<td>0.009±0.0004</td>
</tr>
</tbody>
</table>

The experiment described here is the first attempt at measurements of flux parameters under steady-state conditions in the living animal. The data demonstrate that such experiments are now possible so that issues of regulatory aspects can now be addressed. Since this approach can be applied to any element for which enriched stable isotopes are available, a general method is now available for such studies.

Conclusions. The two examples discussed briefly above indicate the potential for use of stable isotopes in numerous studies of metabolism of inorganic elements. These are now possible in large part because of the recent developments in stable isotope measurement with inductively coupled plasma mass spectrometry. These applications are in their early stages, but the work reported to date illustrates clearly the major contributions that this technique can now make to a fuller understanding of many important issues of mineral metabolism whose exploration is not possible otherwise.

A method for the determination of boron by stable isotope dilution in a variety of biological samples is described. Sample material is fused with sodium carbonate. Boron is separated from matrix components by using Amberlite IRA-743 borate-selective ion-exchange resin. Boron is eluted with 1% HNO₃, and eluates are introduced into an inductively coupled plasma mass spectrometer through a direct injection nebulizer. This nebulizer provides a fast washout of ~15 seconds for residual boron, which greatly decreases the memory effects characteristic of conventional pneumatic nebulizers. \(^{11}\text{B}/^{10}\text{B}\) ratios are determined with relative standard deviations between 0.4 and 1.5%, and the detection limit for boron is approximately 1 ng/g in these samples. Stable isotope dilution methodology for quantitation of boron shows that: (1) fusion of sample with sodium carbonate avoids volatilization of boron from samples; (2) approximately 80% of submicrogram amounts of boron from samples can be recovered from the resin with insignificant isotopic fractionation; (3) results for biological reference materials are in agreement with certified or non-certified values (see Table I); and (4) the boron concentration of pooled human blood plasma is $23 \pm 2 \mu g/L$ (95% confidence interval). Assessment of effects of boron on calcium metabolism for prevention of osteoporosis should be expedited by use of this method. Research supported by United States Department of Agriculture Competitive Research Grants Office, Grant No. 89-37200-4453, and by the Director of Energy Research, Office of Basic Energy Sciences, U.S. Department of Energy. The Ames Laboratory is operated by Iowa State University for the U.S. Department of Energy under contract number W-7405-Eng-82.

Reference:
F.G. Smith, D.R. Wiederin, R.S. Houk, C.B. Egan, and R.E. Serfass, 

Table I. Boron in biological materials.

<table>
<thead>
<tr>
<th>Material</th>
<th>Boron found*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled Human Blood Plasma</td>
<td>$23 \pm 2 \mu g/L$</td>
</tr>
<tr>
<td>NIST SRM 909: &quot;Human Serum&quot;</td>
<td>$26 \pm 6 \mu g/L$</td>
</tr>
<tr>
<td>NIST SRM 1548: &quot;Total Diet&quot;</td>
<td>$2.52 \pm 0.08 \mu g/g$</td>
</tr>
<tr>
<td>NIST SRM 1571: &quot;Orchard Leaves&quot;</td>
<td>$35.5 \pm 0.8 \mu g/g$</td>
</tr>
</tbody>
</table>

* Values reported are Mean ± 2 SD of 3 to 6 replicates.*
MULTIPLE-ISOTOPE DILUTION TECHNIQUES USED WITH MULTIPLE-COLLECTOR, THERMAL IONIZATION MASS SPECTROMETRY TO INVESTIGATE MINERAL METABOLISM IN HUMANS

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The application of magnetic sector, thermal ionization mass spectrometry (TIMS) to stable isotope dilution studies in humans was first reported in the 1970's. Magnetic sector TIMS is considered to be the most precise and accurate method available for measuring isotopic ratios of most mineral elements. A limitation of the method, slow analysis, has recently been minimized by computer-controlled instruments equipped with multiple sample turrets and multiple-isotope collectors. Our TIMS is equipped with 5 variable Faraday collectors for simultaneous collection of up to 5 isotopes of an element. We used this TIMS and enriched stable isotopes in a number of human studies to investigate copper, zinc, and iron metabolism, for limited studies of calcium and magnesium metabolism, and are now using the technique for the analysis of samples from a major study of molybdenum metabolism.

Stable isotopes of up to five elements were administered simultaneously. For studies of zinc, iron, and molybdenum metabolism, one isotope was fed and another infused, as follows:

Fed: $^{67}$Zn, $^{64}$Fe, and $^{100}$Mo
Infused: $^{65}$Zn, $^{68}$Fe, and $^{97}$Mo

The isotopes were prepared for feeding or infusion by first dissolving them in hydrochloric acid. Feeding solutions were diluted further, then added to components of the diet about two hours before meals. Isotopes to be used for infusions were dissolved under sterile conditions. The solutions were prepared for infusion and tested for endotoxins by a pharmacologist. Solutions were infused over a period of two minutes.

Blood, urine, and fecal samples were collected following infusion and/or feeding of isotopes. Blood was drawn 12 times after the isotope infusions to follow disappearance of the isotopes and to develop models of mineral metabolism. Complete urine collections were made following infusions, with 8-hr collections on the days of isotope infusions and 24-hr collections thereafter. Fecal samples were combined into 6-day pools.

With the exception of copper, the amounts of isotopes and elements appearing in a sample were determined by dilution with a second or third isotope of each element prior to sample preparation and analysis. Since copper has only two stable isotopes, the isotopic and total copper contents of a sample were determined by additional isotopic enrichment of a replicate sample.

Samples were ashed and minerals separated and purified using ion exchange chromatography. Isotopic ratios were measured on purified minerals using a magnetic sector, thermal ionization mass spectrometer. Analytical conditions for five minerals are shown in Table 1. Most ratios were measured with internal and external precision of within 0.1% (1).

Multiple-isotope dilution formulas and fractionation corrections were used to calculate isotopic enrichment, absorption, and endogenous excretion of the minerals (2,3). Examples of fecal enrichment following feeding and infusion of isotopes are shown in Table 2. Isotopic enrichments of Zn and Cu in blood plasma and Zn in urine were followed during the course of 80 to 120 day studies following isotope infusions and feedings.

### Table 1. Analytical Parameters (1)

<table>
<thead>
<tr>
<th>Element</th>
<th>Sample Size</th>
<th>Type of Solution</th>
<th>Number of Filaments</th>
<th>Pilot Mass</th>
<th>Normalizing Ratio</th>
<th>Usual Ion Beam Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>2</td>
<td>HNO$_3$</td>
<td>Single*</td>
<td>64, 66</td>
<td>64/68</td>
<td>1.0</td>
</tr>
<tr>
<td>Cu</td>
<td>4</td>
<td>HCl</td>
<td>Single*</td>
<td>63</td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Fe</td>
<td>5</td>
<td>HCl</td>
<td>Single*</td>
<td>56</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>Ca</td>
<td>1</td>
<td>HNO$_3$</td>
<td>Double</td>
<td>40</td>
<td></td>
<td>7.0</td>
</tr>
<tr>
<td>Mg</td>
<td>5</td>
<td>HCl</td>
<td>Double</td>
<td>24, 187</td>
<td>26/24</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* Silica gel ionization enhancement technique used
Table 2. Isotopic ratios and enrichment of fecal samples following infusion and feeding of stable isotopes to young men (1)

<table>
<thead>
<tr>
<th>Isotope Infused</th>
<th>Amount Infused</th>
<th>Ratios Infused</th>
<th>Six day pool following infusion (n=5)</th>
<th>Twelve day pool following feeding (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg</td>
<td></td>
<td>Ratio Average</td>
<td>Range</td>
</tr>
<tr>
<td>70Zn</td>
<td>371</td>
<td>70/68</td>
<td>0.03491</td>
<td>0.03445-0.03533</td>
</tr>
<tr>
<td>56Fe</td>
<td>256</td>
<td>56/56</td>
<td>0.003153</td>
<td>0.00314-0.00317</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ratio Average</td>
<td>Range</td>
</tr>
<tr>
<td>65Zn</td>
<td>8.0</td>
<td>65/68</td>
<td>0.4557</td>
<td>0.3741-0.5181</td>
</tr>
<tr>
<td>65Cu</td>
<td>1.6</td>
<td>65/63</td>
<td>0.5227</td>
<td>0.5041-0.5395</td>
</tr>
<tr>
<td>65Fe</td>
<td>6.3</td>
<td>54/56</td>
<td>0.1230</td>
<td>0.1073-0.1397</td>
</tr>
</tbody>
</table>

Diet contained approximately 15 mg Zn, 1.7 mg Cu, and 10 mg Fe

References

The 99th ASMS Conference on Mass Spectrometry and Allied Topics

Absorption of Dietary Calcium
Using Dual Stable Isotopic Tracers
and Thermal Ionization Mass Spectrometry

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The fraction of dietary Ca that is absorbed can be determined in a number of ways. The classical methodology involves meticulous determination of the Ca content of all foods consumed as well as that contained in urine and feces over a period of 7-9 days - the so-called balance technique. One alternative to this procedure is to adminster an isotope of Ca with meals and use the recovery of isotope dosage as a measure of the fraction of dietary Ca absorbed. This procedure, however would still require the collection of fecal material and would not recognize the degree to which the body excretes Ca into the GI tract, the endogenous fecal excretion. An intravenously administered tracer would surmount this problem, but fecal collection methods are inherently subject to substantial variance. We have developed a technique that will permit the measurement of the fraction of dietary calcium that is absorbed which both results in a lower variance in the measured values and is easier to execute than the balance, or fecal collection techniques.

Two stable isotopic tracers are administered simultaneously, one orally after equilibration with the calcium form in the food. The second tracer is given intravenously. Any of the oral tracer that subsequently appears in urine has of course been absorbed and then excreted. By making a complete urine collection over a 24 hour period and determining the total amount of each of the tracers that is present, one is able to calculate the fraction of the dietary calcium that has been absorbed. That is to say, \( \alpha \), the fraction of dietary calcium absorbed is given by:

\[
\alpha = \frac{t_{\text{oral tracer in urine}}}{t_{\text{IV tracer in urine}}} \quad (1)
\]

when both numerator and denominator are corrected for dosage.

The major assumptions of this equation are that the isotope in the gastrointestinal tract is an accurate reflection of the movement of natural calcium moving from there to the circulation and that, once in the circulation, the oral tracer's behavior is exactly the same as the intravenously administered material. The denominator of Equation 1 is simply a normalization to account for this latter assumption. A closely related expression can be written for the calculation of \( V_{\text{endo}} \). It is based on the assumptions that any of the intravenous tracer that appears in feces has been excreted there without subsequent resorption, and that the ratio of the fraction of intravenous tracer dose in stool to urine is equal to the ratio of the rates at which Ca flows to the gastrointestinal tract, \( V_{\text{endo}} \), and urine, \( V_\text{u} \),

\[
V_{\text{endo}} = \frac{t_{\text{IV tracer in feces}}}{t_{\text{IV tracer in urine}}} \quad (2).
\]
If the experiment is done by taking a series of urine samples over the first 24 hours after administration, then one could calculate the time course of $\alpha$. The orally administered tracer would be seen to be initially at zero in urine, rise, pass through a maximum and then begin to fall. The rate of disappearance of this tracer of course mimics the disappearance of the intravenously administered tracer, once the absorption process is complete. The ratio of the two areas under curves, Eq. 1, takes the shape of a function, $y = 1 - \exp(bt)$, and approaches the final or true value of $\alpha$ only asymptotically. It can be shown that in a 24 hour study, the value of $\alpha$ that is reached is within 5-7% of the asymptotic value.

Comparison of the results of our dual tracer technique with those of a metabolic balance study carried out simultaneously have shown that while the same mean value is obtained for the amount of calcium absorbed, $0.26 \pm 0.09$ by tracers and $0.26 \pm 0.09$ by balance. The tracer technique results in a 30% reduction in the variance of the mean. The consequence of this is that one is able to detect smaller changes in absorption with the tracer methods than with balance techniques.

In a group of 12 premature infants, mean gestational age: 31±2 wks, mean age at study: 20±10 days, mean weight at study: 1693±174 gm, true absorption was 57±16% of ingested calcium and net retention was 48±18% of dietary calcium. The most interesting aspect of this study was that $V_{\text{endo}}$ was 7±4% of intake, a value 5-6 greater than seen in healthy older children and adults. In a study of 7 adolescent girls with anorexia nervosa, we found that their absorption was 15±4% of intake, down about a factor of 3 from healthy adolescent girls. Of considerable interest in addition is that we observed that the girls with anorexia excreted almost twice as much calcium in their urine than did the healthy girls ($234\pm68$ mg/day vs $140$ mg/day max). The consequence of the low absorption and high urinary excretion for the patients with anorexia is that they are in negative calcium balance of $93\pm106$ mg/day compared with the positive balance of between 100-400 mg/day seen in healthy girls. In a series of studies of a woman in her third trimester of pregnancy, during lactation and then as a self-control, we have determined that due to changes in Ca absorption and urinary excretion $[\alpha = 0.33_{\text{preg}} \text{ vs } 0.19_{\text{cont}}, V_u = 9.1 \text{ mg/hr preg} \text{ vs } 5.8 \text{ mg/hr cont}]$ during pregnancy lead to an average accumulation of 5.9 g Ca during the 3rd trimester, presumably to the fetal skeleton. Since the fetus accumulates about 24g Ca during this trimester, the maternal skeleton contributes about 18 g Ca. A similar set of comparisons during lactation show little change in $\alpha$, 0.22 vs 0.19, and a net retention of urinary Ca, 1.7 mg/hr vs 5.8 mg/hr, coupled with estimated losses of Ca in milk of about 12.9 g over a 6 month period of nursing. These two losses combined lead to an estimated loss of maternal skeletal Ca of 39g, or about 4% of the maternal skeleton. Such losses clearly cannot be irreversible, and one goal of future studies is to quantify the 'make up period' of the maternal skeleton. The wide range of applicability of this technique to populations of different ages and physiological states suggests that it has the potential for substantially increasing our knowledge of calcium utilization.
Isotopic substitution for tracer purposes is among the most powerful techniques available for elucidation of metabolic pathways and general molecular physiology. Tracer studies using radioactive nuclides is recognized as highly sensitive, but is of increasingly limited applicability for human studies due to the health risks to subjects attendant to radioactivity. Risks of exposure to research workers and particularly the problem of radioactive disposal further restricts radiolabel use.

Since they present none of the health concerns, stable isotopic tracers are an obvious alternative to radiotracers, but are considered to be of less utility because of ubiquitous background levels of the minor isotope. However, the background level of minor isotope does not limit detectability; rather, the stability of background levels and the precision with which they can be determined are of fundamental importance. In general, $^{13}$C levels vary at over a range 2-3 orders of magnitude lower than the precision available by full scan GC/MS analysis. Measurements at a higher level of precision should result in a commensurate increase in detectability.

Gas chromatography/combustion/mass spectrometry (also called “Isotope Ratio Monitoring/GC” (IRM/GC) and GC Isotope Ratio Mass Spectrometry (GC/IRMS)) has recently been introduced commercially as a means for achieving high precision for carbon isotope ratios of compounds emerging from a GC. In addition to this development, technology for the production of highly enriched, perlabeled (uniformly labeled, [U-$^{13}$C] and/or D) biomolecules from algae has emerged. Algal biomasses are grown in closed loop incubators with $^{13}$CO$_2$ and/or D$_2$O are capable of producing enrichments $\geq$ 99% $^{13}$C. Our preliminary results indicate that the combination of these two approaches permit tracer experiments with superior sensitivity to radiotracers.

Analytical Figures-of-Merit We have evaluated the accuracy and precision of GC/C/MS system based on a Finnigan MAT 252 and Varian GC. Replicate determinations of an equimolar mixture of $C_{14-16}$ hydrocarbons yield standard deviations (SD) $\approx 0.2\%_o$. Replicates of a calibrated fatty acid methyl ester (FAME) mixture over a factor of 200 quantity injected yield SD $\approx 1\%_o$. Accuracy for these samples calibrated against CO$_2$ revealed systematically lower values. However, calibration against an internal standard yields accuracy for strong peaks (1 nanomole) within 0.15% $\%_o$.

Human Tracer Experiment To provide a live human experimental test of the techniques, an experiment designed to test specific observation regarding human fatty acid interconversion was performed. There exist several lines of evidence which suggest that stearic acid (18:0) is converted to oleic acid (18:1) in the liver but not in the intestine.
Ten mg of 42% $^{13}$C labeled 18:0 were administered orally to humans. Blood was sampled hourly for 10 hours and daily for 14 days. Plasma was prepared, then chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL), and D > 1.21 were prepared by ultracentrifugation. Samples were saponified, acidified, fatty acids were extracted and methylated for GC/C/MS injection. Samples were injected in duplicate.

Figure 1 is a plot of the fatty acid enrichments in chylomicrons for one subject as a function of time. The highest enrichment in this spectrum is 180 %, which occurs at 10 hrs. We believe this enrichment level, strongly discerned by the current instrument, would be indistinguishable from background in a single ion monitoring full scan GC/MS instrument. The background levels of 14:0 and 18:2 vary by ≤ 2 permil through the period. We also observe no deviation from background for the 18:1 signal, indicating no conversion of 18:0 to 18:1 in intestine. Some evidence for conversion in the liver was found in the VLDL fraction.

Based on these data, we estimate that the dose of radiotracer required to produce a similar signal-to-noise ratio to that observed here is 10 μCi. this level is significant and would pose a significant health risk to human subjects.

![Chylomicron Fatty Acids](image-url)
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

PBEIMS AND PBCIMS FOR THE ANALYSIS OF DOXORUBICIN, DAUNORUBICIN AND CARMINOMYCIN

J. Bloom, P. Lehman, W. A. Korfmacher, M. Israel, O. Rosario.

INTRODUCTION:
Particle beam (PB) is one of the more recently developed interfaces for the coupling of HPLC and MS. A major advantage of this interface is the generation of either conventional electron impact (EI) or chemical ionization (CI) mass spectra. The change between EI and CI can be performed without any modification of the HPLC system. HPLC/PBMS can be used in the analysis of thermally labile and nonvolatile compounds such as anthracyclines.

Anthracyclines are very powerful anticancer and antibiotic drugs. Two of these compounds, doxorubicin and daunorubicin, have been extensively used for the treatment of neoplastic diseases in man. In addition, carminomycin is another anthracycline under current study. Typically, HPLC with different detection methods such as UV, fluorescence and electrochemical detection, has been utilized for the analysis of these compounds. Even though these techniques are very valuable for the detection of these compounds, none of them provide structural information.

The utility of PBMS in both EI and CI (positive and negative ion modes) was investigated for the characterization of these three anthracyclines. Characteristic fragment ions can be used for the detection of these compounds via HPLC/PBMS.

EXPERIMENTAL:
The standards were doxorubicin and daunorubicin (Sigma, St. Louis, MO). Carminomycin was obtained from M. Israel. All standards were dissolved in methanol at a concentration of 0.3-0.5 mg/ml.

The PBMS analysis was performed on a Hewlett-Packard 5988A quadrupole mass spectrometer. The EI mass spectra were acquired at 70 eV, with a source temperature at 250 °C. The CI mass spectra were obtained in the positive and negative ion modes using methane as the reagent gas adjusted to 0.50 torr and an analyzer pressure of 2.1 x 10⁻³ torr. The particle beam (Hewlett-Packard 59980A) interface desolvation chamber was held at 65 °C. The helium nebulizer pressure was set at 35 psi. The mobile phase used was 50/50 acetonitrile/water, 0.2 M ammonium acetate, pH 4.5) delivered at a flow rate of 0.5 ml/min from a Hewlett-Packard 1090M HPLC system employing a 10 μL loop. The introduction of the sample to the interface was via direct injection.

RESULTS AND DISCUSSION:
When analyzed by PB under EIMS conditions, the mass spectra for these compounds included a molecular ion that was either of very low intensity or not observed at all. For all anthracyclines, the base peak corresponded to the ion formed after cleavage at the glycosidic bond, and another cleavage at the C(9) position with the loss of the side chain. In addition to the base peak, ions corresponding to additional fragmentation of these compounds were also seen.

The methane PBCI mass spectra, in the positive ion mode, for carminomycin is shown in figure 1. Under these conditions, only a weak protonated molecule was observed for carminomycin and daunorubicin, whereas for doxorubicin it was not seen. The fragment ion at m/z 369 is assigned to the cleavage at the glycosidic bond with retention of the oxygen atom by the aminosugar, i.e. the [MH - Sugar(OH)]⁺ ion; as was also observed for the other anthracyclines. The fragment ion at m/z 349 is assigned to the [MH - Sugar(OH) - H₂O]⁺ ion; which is also seen for daunorubicin. The relative ratios of the ions for these compounds differed greatly among them.
When analyzed by methane PBCIMS using negative ion detection, all three anthracyclines showed significant M' ions. For doxorubicin, this ion was the base peak, and for carminomycin the same ion had a high relative intensity (95%) as we can observe in figure 2. On the other hand, the relative intensity of the M' ion for daunorubicin was 30%. For daunorubicin and carminomycin, the base peak corresponded to the aglycone anion.

CONCLUSIONS:
Significant fragment ions are observed when PBCIMS under both positive and negative ion modes is used for the detection of these anthracyclines. The ions seen are similar to those reported in the literature when other MS techniques are used for their analysis.

When comparing PBEIMS and PBCIMS, the use of the latter for the analysis of these compounds is preferred, because the spectral data obtained via PBCIMS was of greater utility. In general, negative ion analysis provided ions of greater intensity in the molecular ion region for all three compounds when compared to the positive ion mode.

The results obtained by PBCIMS are similar to those reported by Smith (2) under methane DCIMS conditions, although Smith (2) reported that the M' ion was the base peak for daunorubicin and carminomycin.

REFERENCES:
UTILITY OF TSMS FOR THE CHARACTERIZATION OF THREE ANTHRACYCLINES

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¹National Center for Toxicological Research, Jefferson, AR 72079 U.S.A.; ²Department of Chemistry, University of Puerto Rico, Rio Piedras, P.R. 00931, ³Department of Pharmacology, Health Science Center, University of Tennessee at Memphis, Memphis, TN 38163 U.S.A.

INTRODUCTION:

Anthracyclines are very powerful antitumor antibiotic drugs; two of them, doxorubicin and daunorubicin, have been extensively used for the treatment of neoplastic diseases in humans (1). In addition, carminomycin is another anthracycline currently being investigated. Typically, the methods of analysis for these compounds have utilized HPLC with UV, fluorescence or electrochemical detection. These are valuable techniques, but they do not provide structural information.

Thermospray mass spectrometry (TSMS) in both positive and negative ionization modes was used in the characterization of these three anthracyclines. The structural information obtained by this analytical technique, such as the characteristic fragment ions, can be applied to the detection and identification of these compounds by HPLC/TSMS.

EXPERIMENTAL:

The standards were doxorubicin and daunorubicin (Sigma, St. Louis, MO). Carminomycin was obtained from M. Israel. All standards were dissolved in methanol at a concentration of 0.3-0.5 mg/ml.

The TSMS analysis was performed on a Finnigan-MAT TSQ-70 triple quadrupole mass spectrometer using positive and negative ion detection, in the filament-off and discharge-off mode. Typically, the vaporizer temperature was set to 80 °C and the block (aero) temperature to 250 °C. Introduction of the sample was via direct injection using an ISCO LC-5000 syringe pump with a Rheodyne 7125 injector equipped with a 20 μl loop at a flow rate of 1.25 ml/min. The mobile phase used was 50/50 acetonitrile/(water, 0.2 M ammonium acetate, pH 4.5).

RESULTS AND DISCUSSION:

The positive ion TS mass spectrum for daunorubicin in figure 1 shows a protonated molecule ([MH]+' at m/z 528 with a relative intensity of 65%. The fragment ion at m/z 383 is assigned to the cleavage of the glycosidic bond with the oxygen atom retained by the aminosugar, i.e. the [MH - Sugar(OH)+' ion. The fragment ion at m/z 363 is assigned to the [MH - Sugar(OH) - H₂O]+' ion. For the fragment ion at m/z 337, this was assumed to be due to the cleavage of the glycosidic bond and the loss of the side chain at the C(9) position.

A protonated molecule and characteristic fragment ions were also observed for the other anthracyclines. The [MH]+' ion for doxorubicin had a relative intensity of 20% while the same ion for carminomycin was the base peak. The base peak for doxorubicin was observed at m/z 337, which was assigned to the cleavage at the glycosidic bond and the loss of the side chain. The [MH - Sugar(OH)+' ion for doxorubicin and carminomycin were observed at m/z 397 and m/z 367, respectively.

When analyzed by TSMS using the negative ion mode, all three anthracyclines produced intense [MH]+' ions. For daunorubicin (see figure 2) and carminomycin this ion was the base peak; while for doxorubicin this ion had a relative intensity of 40%. The base peak for doxorubicin is observed at m/z 335 which is assumed to be the aglycone anion with a loss of the side chain.

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In Figure 2, we observed the fragment ion at m/z 397 which is assumed to be due to the cleavage of the glycosidic bond with a retention of the oxygen atom by the aglycone; the same loss is seen for doxorubicin and carminomycin at m/z 413 and m/z 383. In addition, all three anthracyclines showed fragment ions at m/z 377, m/z 361, and m/z 347 for doxorubicin, daunorubicin and carminomycin, respectively, due to a cleavage of the glycosidic bond and the loss of H₂O.

CONCLUSIONS:
Under TSMS in the positive ion mode, all three anthracyclines displayed a protonated molecule and an [M-H - Sugar(OH)]⁺ ion. The relative ratios of these ions differed significantly for these compounds, and additional minor peaks were also observed.

For the TSMS negative ion mode, the [M-H]⁻ ion was the major ion for two of the compounds. Characteristic fragment ions were observed for all of the anthracyclines. In general, negative ion analysis provided an ion in the molecular ion region of greater relative intensity than the one obtained under positive ion mode.

REFERENCES:
DETERMINATION OF NICOTINE AND ITS METABOLITES IN SMOKERS' URINE
BY THERMOSPRAY LC/MS

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R. J. Reynolds Tobacco Co., Pharmacology Research
Bldg. 630-2, Winston-Salem, NC 27102.

Nicotine disposition in smokers has been studied for decades and yet the ability to account for all of the nicotine absorbed by smokers has been difficult. The relatively recent determination of a major metabolite, trans-3'-hydroxycotinine and the reports of glucuronide conjugates of nicotine, cotinine, and trans-3'-hydroxycotinine [1-3] has significantly improved the potential for quantifying nicotine uptake in smokers. Some of these metabolites are thermally labile or difficult to extract from urine which hinders quantification. In this study, thermospray LC/MS was used to profile urinary metabolites of nicotine in smokers by direct injection of urine.

The method used is a modification of a reported technique that provides for the direct injection of urine onto an LC system connected via a thermospray interface to a quadrupole mass spectrometer [4]. All nonconjugated metabolites were monitored in a single chromatographic run without extraction or derivatization of the analytes. Separation was performed on a polymer column with a water/methanol gradient and ammonium acetate buffer was added post-column using a reversed-gradient system. The thermospray source was operated in the positive ion, filament-off mode and the (M+H)^+ ions were detected by selected ion monitoring. Quantification was done by the method of internal standards using d₆-cotinine as the internal standard. Limits of detection varied from approximately 20 to 200 ng/mL for the analytes in this study.

Urine samples were collected from 11 normal male smokers and analyzed. The method screens for nicotine and 17 of its metabolites but only six analytes were consistently found in smokers' urine: nicotine, cotinine, trans-3'-hydroxycotinine, nicotine-N'-oxide, cotinine-N-oxide, and demethylcotinine. Glucuronide conjugates of nicotine, cotinine, and trans-3'-hydroxycotinine were determined by treating extracted urine samples with β-glucuronidase and quantifying the aglycones by LC/MS. Table 1 summarizes the average results for the smokers in μg per 24 hr period. The variability (CV) in absolute amount among the nine analytes ranged from 35 to 70%. The major metabolite in all smokers' urine was free trans-3'-hydroxycotinine which accounted for an average of 35% of total metabolites measured. Conjugates constituted an average of 29% of the total metabolites measured. The average distribution of metabolites for these smokers is plotted in Figure 1 for the free and conjugated metabolites. These values are normalized by using the percent of total metabolites determined for each smoker. Although it has not been demonstrated that all of the nicotine absorbed while smoking can be quantitatively recovered in urine as nicotine and its metabolites, it is of interest to determine what portion of FTC nicotine yield can be recovered in urine. FTC nicotine input was determined from the number and type of cigarettes consumed by each subject over the 24 hour period. The nicotine output was determined from the total nicotine equivalents measured in this study. The ratio of nicotine metabolic output to FTC nicotine input for these 11 smokers ranged from 0.58 to 1.44 with an average of 0.96 ± 0.29.

This study shows that thermospray LC/MS can be successfully applied to survey urinary nicotine metabolite distribution in smokers. The data in this study demonstrate that a significant percentage of nicotine absorbed while smoking can be accounted for in the urine as nicotine, cotinine, trans-3'-hydroxycotinine, and their glucuronide conjugates. This method confirms the presence of glucuronide conjugates of nicotine, cotinine, and 3'-hydroxycotinine which constitute an average of 29% of the urinary metabolites.

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TABLE I

Average amount of urinary nicotine and its metabolites excreted by 11 smokers during ad libitum smoking over 24 hours.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>µg per 24 hr ± st. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-3'-Hydroxycotinine (free) [3HC]</td>
<td>9239 ± 5093</td>
</tr>
<tr>
<td>trans-3'-Hydroxycotinine (conjugated) [3HC-G]</td>
<td>2313 ± 1600</td>
</tr>
<tr>
<td>Cotinine (free) [COT]</td>
<td>3111 ± 1392</td>
</tr>
<tr>
<td>Cotinine (conjugated) [COT-G]</td>
<td>3601 ± 1465</td>
</tr>
<tr>
<td>Nicotine (free) [NIC]</td>
<td>2269 ± 1539</td>
</tr>
<tr>
<td>Nicotine (conjugated) [NIC-G]</td>
<td>560 ± 392</td>
</tr>
<tr>
<td>Nicotine-N'-oxide [NNO]</td>
<td>1497 ± 489</td>
</tr>
<tr>
<td>Cotinine-N-oxide [CNO]</td>
<td>1003 ± 674</td>
</tr>
<tr>
<td>Demethylcotinine [DMC]</td>
<td>331 ± 116</td>
</tr>
</tbody>
</table>

FIGURE 1

AVERAGE DISTRIBUTION OF URINARY NICOTINE METABOLITES IN 11 SMOKERS

References


Predicting Thermospray LC/MS Fragmentation of Glucuronides By Molecular Modeling
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Introduction: Thermospray LC/MS analysis of glucuronide conjugates can produce intact pseudomolecular ions or aglycone ions. In this analysis a series of substituted phenol glucuronides were systematically analyzed by thermospray mass spectrometry under different thermospray ion source interface conditions and detection modes. The substituted phenol glucuronide conjugates were also analyzed by molecular modeling techniques to provide a systematic measurement of the effects the substitution pattern had on the observed thermospray spectra. A comparison of the effects of phenol ring substituents and the effects of the interface parameters suggested that the spectra obtained by thermospray LC/MS depended more on the molecular structure of the analyte than the interface parameters.

Materials and Methods: Thermospray Analyses: LC/MS spectra were obtained on a Nermag R 30-10 triple stage quadrupole mass spectrometer using the Nermag thermospray source without discharge or filament assisted ionization. The glucuronides were analyzed at source block temperatures of 200°C, 250°C, and 300°C and at ion source repeller voltage settings of 100, 150, 200 and 300 electron volts. The tip temperature was optimized at 224°C for the 200°C block temperature analyses and at 230°C for the 250°C and 300°C block temperature analyses. Molecular Modeling Analysis: All calculations were performed using Sybyl and MOPAC. The lowest energy conformer for each was then submitted to quantum mechanics for geometry optimization using the AM1 Hamiltonian. Analysis of the quantum mechanics data focused on evaluations of bond strength and ease of bond scission. Two measures were tabulated: bond order (calculated from MOPAC/AM1) and a “bond polarity index” represented by the arithmetic difference between the partial atomic charges for the bonds of interest.

Results: Phenyl-b-D-glucuronide produced a major ammoniated pseudomolecular adduct ion at m/z=288 upon thermospray ionization (Figure 1). Electron donating analogs, 4-ortho-amino-phenyl-b-D-glucuronide (Figure 2) and p-amino-thio-phenylglucuronide (Figure 3) produced robust pseudomolecular ions. However, the spectrum of p-nitro-phenol glucuronide, an electron withdrawing analog, produced an intense aglycone ion at m/z=137 (Figure 4).

The lack of thermospray interface parameter effects (ion source block temperature and repeller voltage) on the observed fragmentation suggested that the fragmentation results were a function of the intrinsic glucuronide structure rather than the thermospray ionization conditions. Thus, a molecular modeling evaluation of each glucuronide was performed (Table 1). In each case, the O-Gluc bond order is numerically less than the Ar-O bond order. Experimentally, Ar-O bond scission has not been seen - only O-Gluc scission in mass spectrometry fragmentation patterns. Therefore quantum mechanical bond order can perhaps be used as an indication for bond fragmentation. Additionally, the degree of O-Glucuronide bond scission (versus intact glucuronide and other bond fragmentation in the molecule) appears to correlate well with the “bond polarity index” as calculated from partial atomic charges. The unsubstituted phenyl glucuronide yielded a mixture of intact glucuronide and the aglycone while the p-nitro yielded primarily the aglycone. The o-amino and p-amino(S) substituted phenyl glucuronides compounds produced primarily the intact glucuronide. It appears that scission of the O-Gluc bond increases as the “bond polarity index” increases. When the analysis was attempted on two other glucuronides that were not in the substituted phenol class, the predicted thermospray mass spectrometric fragmentation based on their bond order and bond polarity index was observed.

The “bond polarity index” of 4-methylumbelliferyl-b-D-glucuronide predicted that an intact molecular ion would be produced. The thermospray spectrum of 4-methylumbelliferyl-b-D-glucuronide was characterized by a pseudomolecular ion at m/z=353 and a glucuronic acid ion at m/z=177 (Figure 5). The “bond polarity index” of a-naphthyl glucuronide also predicted that an intact molecular ion would be produced. The thermospray spectrum of a-naphthyl glucuronide revealed a pseudomolecular ion at m/z=343 as well as the natriated adduct at m/z=365 (Figure 6).

These data suggest that the o-glucuronide bond strength should allow one to predict the fragmentation of a particular glucuronide in thermospray ionization. Thus, an examination of the electron density of a particular glucuronide could assist the mass spectrometrist in selecting the optimal technique for its analysis.
Table 1 AM1 Quantum Mechanics Calculated Values

<table>
<thead>
<tr>
<th>Glucurono</th>
<th>O-Gluc Bond Order</th>
<th>Ar-O Bond Order</th>
<th>O-Gluc BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>unsub</td>
<td>0.9552</td>
<td>0.9917</td>
<td>0.5728</td>
</tr>
<tr>
<td>p-nitro</td>
<td>0.9395</td>
<td>1.0214</td>
<td>0.6006</td>
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<td>o-amino</td>
<td>0.9740</td>
<td>0.9947</td>
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<td>a-naphth.</td>
<td>0.9739</td>
<td>0.9889</td>
<td>0.3193</td>
</tr>
<tr>
<td>p-amino(S)</td>
<td>0.9511</td>
<td>0.9897</td>
<td>0.3070</td>
</tr>
<tr>
<td>umbillic</td>
<td>0.9668</td>
<td>0.9957</td>
<td>0.3214</td>
</tr>
</tbody>
</table>
Identification of Nizatidine Degradation Products by LC/MS/MS and FAB/MS/MS


Indianapolis, IN 46285

Introduction: Nizatidine is a histamine H-2 receptor antagonist that blocks the gastric H-2 receptor, leading to inhibition of gastric acid secretion from the parietal cells of the stomach. While the toxicity of this class of compounds is quite low, it has been observed that ranitidine (1), cimetidine (2-4), and famotidine (5) form nitrosamines subsequent to degradation by nitrous acid. The nitrosated products of cimetidine (2-4), famotidine (5) and ranitidine (6-7) have been reported to be positive in bacterial mutation assays. However, in vivo carcinogenic tests with cimetidine, famotidine, and ranitidine have been negative. In this paper we have explored the reactivity of nizatidine with nitrous acid and the mutagenicity of the resultant product mixture. Mixture analysis by thermospray LC/MS led to the identification of four major reaction products (Figure 1). The identification of these reaction products was confirmed by high resolution fast atom bombardment mass spectrometric analyses, spectral comparison to known reference standards (when available) and daughter ion collisional induced dissociation (CID) spectra.

Degradation experiments utilizing 15N sodium nitrite/HCl followed by LC/MS/MS and FABMS analyses indicated that only one out of the four observed degradation products incorporated 15N (Figure 2) leading to the conclusion that nizatidine does not form nitrosamines upon degradation by nitrous acid. The mixture of nizatidine degradation products did not produce revertant mutations in the bacterial mutation assays utilizing S. typhimurium or E. coli.

Methods: Reaction Conditions

Nizatidine (2.5 g, 7.55 mmole) and sodium nitrite (2.1 g, 30.4 mmole) were added with stirring to water (760 ml) at 37°C in a 3-neck flask. Hydrochloric acid (20 ml of 1N) was added until pH 3.68 was achieved. The solution was stirred for 3 hours at 37°C. Ammonium sulfamate (4.33 g, 38.0 mmole) was added to consume the excess nitrous acid. The solution was lyophilized and stored at approximately -20°C.

LC/MS

Twenty five microliters (50 ug) of the sample dissolved in mobile phase was injected through a Rheodyne injector onto a 15 cm, 5 micron, Jones ODS reverse phase HPLC column. The mobile phase consisted of 760 ml of an 0.1 M ammonium acetate buffer (adjusted to pH 7.5 with glacial acetic acid) and 215 ml methanol. The isocratic separation was achieved by pumping mobile phase at a flow rate of 1 ml/min of mobile through the column using a Waters 600-MS HPLC pump. The HPLC eluent was delivered to a Nermag R 30-10 triple stage quadrupole mass spectrometer equipped with a Nermag thermospray ion source. The ion source block temperature was maintained at 223°C with a probe tip temperature of 206°C. Ammonium sulfamate (4.33 g, 38.0 mmole) was added to consume the excess nitrous acid. The solution was lyophilized and stored at approximately -20°C. LC/MS Twenty five microliters (50 ug) of the sample dissolved in mobile phase was injected through a Rheodyne injector onto a 15 cm, 5 micron, Jones ODS reverse phase HPLC column. The mobile phase consisted of 760 ml of an 0.1 M ammonium acetate buffer (adjusted to pH 7.5 with glacial acetic acid) and 215 ml methanol. The isocratic separation was achieved by pumping mobile phase at a flow rate of 1 ml/min of mobile through the column using a Waters 600-MS HPLC pump. The HPLC eluent was delivered to a Nermag R 30-10 triple stage quadrupole mass spectrometer equipped with a Nermag thermospray ion source. The ion source block temperature was maintained at 223°C with a probe tip temperature of 206°C and a repeller voltage of 150 eV. LC/MS/MS The second quadrupole, Q2, was set as an rf only collision cell that was maintained with a gas collision pressure of 2.5 x 10^-2 torr Argon. The third quadrupole, Q3, scanned the collision induced dissociation fragments at a rate of 1 scan/second. The accelerating potential of the collision was increased by -40 eV to increase the internal energy of the isolated daughter in order to enhance the collision process. FABMS Samples were dispersed in "magic bullet" and subjected to bombardment with 8 keV Xenon atoms for spectra determined on a VG ZAB-3F triple sector (B1EB2) mass spectrometer. For spectra determined on a VG ZAB-2SE double focusing mass spectrometer the dispersed samples were bombarded with cesium ions having a net energy of 12 KeV. Mutation Assay The nizatidine nitrosation reaction mixture was tested for the induction of bacterial mutations using S. typhimurium strains TA1535, TA1537, TA98, and TA100 and Escherichia coli strain WP2uvrA according to published methods (8).

Results and Discussion: Triplicate analysis of three separate nizatidine nitrosation reactions consistently revealed four major products that eluted within 9 minutes (Figure 2) on the reverse phase HPLC system described in the methods section. The assigned structures were supported by high resolution FAB/MS analyses. Since the initial purpose of this work was to identify nitrosated products of nizatidine, a concerted effort was made to look for products that incorporated NO. The search in positive and negative ion detection thermospray LC/MS for an ion at m/z=361 (331 + NO), which would correspond to the pseudomolecular ion of a proposed N-nitrosated nizatidine, did not reveal the presence of N-nitrosated nizatidine. A further attempt to detect the incorporation of NO into nizatidine involved repeating the nitrosation reaction with 15N nitrite followed by LC/MS analysis for the corresponding mass assignment shifts. The shift from m/z=160 to m/z=161 was the
only difference observed in the reaction of nizatidine with \(^{15}\)N nitrite versus \(^{14}\)N nitrite. The LC/MS/MS CID spectrum of the sole reaction product that incorporated \(^{15}\)N suggested that the \(^{15}\)N incorporation occurred at the N-OH moiety (Figure 3). Since no nitrosamines were detected and only 1 reaction product was shown to incorporate NO, the entire reaction mixture was assessed for mutagenic potential in the Ames Salmonella/mammalian microsome assay. It was concluded that the nizatidine nitrosation reaction mixture was not mutagenic either with or without metabolic activation by mammalian microsomes in Salmonella or E. coli.

References:
Continuous Flow FAB/MS for Monitoring Isophosphamide in Human Blood Serum

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Cyclophosphamide (CP) and in particular the less toxic Isophosphamide (IP) are most important as chemotherapeutic drugs in the treatment of cancer and tumors. In the course of our studies on the detection and the chemical reactions of Cyclophosphamide and, especially, Isophosphamide, we have developed an essay for the quantitative determination of those drugs in human blood serum based on interfacing microHPLC with continuous flow FAB/MS (CFFAB/MS).

A rather straightforward clean up scheme for Isophosphamide has been developed with nearly 100% recovery. 250 µL plasma are transferred into a 2 mL test tube and 500 µL icecold acetone were added for precipitation of proteins. After centrifugation the supernatant is decanted into a fresh tube, the remaining proteins are extracted again with 250 µL acetone and the combined solutions are blown to dryness. The residues are redissolved in 250 µL tridistilled water, centrifuged again and from that solution, 20 µL were injected onto a) a micro HPLC column (RP-18, 1.0 x 100 mm) or b) a short semi-microcolumn (RP-18, 1.6 x 20 mm). Since the drug is dissolved in water, a peak compression takes place. The solvent was methanol/water/glycerol 50/40/10 (v/v/v) and a typical flow of 12 µL/min was used for chromatography of the drug and for delivery to the tip of the CFFAB probe.

For this study, we have modified the continuous flow FAB probe (“Bioprobe”) of a Finnigan MAT 90. The standard tip was divided into two units - a base and a removable cylinder.

![Fig. 1: Structures of Cyclophosphamide and Isophosphamide](image)

![Fig. 1: Calibration function and typical range of plasma concentration](image)
with the FAB target surface - to allow for quick exchange of the target. Two different target types were tried, one with an additional drain capillary from exit of the interface capillary to the wick beneath the target, the other with a screen type surface and both gave essentially the same results with respect to stability of the ion current and sensitivity. For the determination of the drug the instrument was used either in scanning mode or with selective ion recording for improved detection limits, which are in the range of 10 ng absolute or 500 ng/ml plasma.

Since the method has been developed with clinical routine application in mind, which means rather high biological and individual variation at any rate, we have tried to avoid the inclusion of an internal standard. We have been able to obtain such a reproducibility in our day to day measurements (standard deviation in the range of 10%) that, considering the necessary accuracy, our results are sufficient. The calibration function obtained with standard solutions is linear over the concentration range of interest (Fig.2).

The described procedure has been used successfully for monitoring the levels of Isophosphamide in blood serum administered to patients over an extended time period, normally 7 days during one ambulant treatment, and we have found concentrations of 50 ug/mL Isophosphamide (Fig.2). A typical behaviour of the drug level in serum is shown in Fig.3. After the first administration the drug accumulates to some extend and remains at rather high levels, whereas the concentration decreases rapidly after the last dose and falls under our detection level. In particular the last result is important, since the toxic side effects of the drug is always of concern. In the moment attempts are under way to include the most important metabolites and catabolites, if necessary, in the study in order to get more complete picture of the behaviour of the cytostatic drug.

Fig.2: Concentration of IP during treatment of a patient for 7 days

Financial support by the "Bundesminister für Forschung und Technologie" and by the "Ministerium für Wissenschaft und Forschung des Landes NRW" is gratefully acknowledged.
METABOLIC INVESTIGATION OF THE SYNTHETIC ANTI-COAGULANT PEPTIDE MDL 28,050 USING CONTINUOUS FLOW FAB LC/MS

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MDL 28,050 is a synthetic decapeptide modeled after the C-terminus of the leech protein hirudin. This molecule, whose structure and sequence appear in Figure 1, is being considered as a clinical candidate due to its anti-coagulant and anti-thrombotic properties. Recently, studies were undertaken to determine the in vivo biotransformation of this molecule in rat. Rats were administered a 9 mg/kg iv bolus dose containing tritiated MDL 28,050 labeled at tyrosine (specific activity 14,270 dpm/ug) and urine was collected at 6 hr intervals. Following initial clean-up by C18 solid phase extraction of a composite 0-24 hr urine sample, the extract was profiled by HPLC using radiodetection. In a second experiment, a 0-6 hr urine sample was extracted and the resultant mixture subjected to LC/MS analysis using CF-FAB.

The details of the system used for LC/MS have recently been published¹ and incorporates on-line UV detection with coaxial flow post-column matrix addition. The packed capillary column (250 mm x 0.32 mm) was slurry packed in-house with 5 μm Spherisorb C18. An aliquot of the urine extract, estimated to contain about 750 ng of total peptide, was injected on-column and eluted with a linear gradient of 5-60% ACN in water (0.1% TFA) over 40 min. at 2 μl/min.

Figure 2 contains a series of mass chromatograms for all the MDL 28,050-related peptide products observed by LC/MS. Two positions: Glu-7Glu and Ile-3Pro were implicated as sites of primary hydrolysis based on the presence of the complementary peptide pairs m/z 847/501 and 621/727, respectively. It is noteworthy that C-terminal peptide metabolites, such as Cha-E-OH (m/z 301), were not detected by radioprofiling due to the location of the tritium label. The data also indicate that MDL 28,050 can be detected in urine 6 hr after dose administration.

Other advantages of LC/MS are illustrated in Figure 3 which compares FAB mass spectra obtained for the N-terminal hexapeptide metabolite by (A) off-line fraction collection and (B) CF-FAB. The spectrum acquired off-line gave (M+Na)⁺ as the dominant species and yielded limited fragmentation. In contrast, the spectrum obtained by CF-FAB gave primarily (M+H)⁺ as well as abundant sequence data which enabled the entire sequence of the peptide to be derived from an amount estimated to be 280 pmol.


Figure 1

Succinyl-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-oGlu-OH
M.W. 1328
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

**Figure 2**

**Figure 3**
ON-LINE AND OFF-LINE MICRODIALYSIS/CF-FAB MS AND GC/MS IN STUDIES OF IN VIVO AND IN VITRO VALPROIC ACID INDUCED BRAIN NEUROCHEMICAL CHANGES

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Valproic acid is widely used in the management of many forms of epilepsy and seizure disorders. However, the mode of drug action in the brain remains unknown. In this study, various mass spectrometric methods were used to study pharmacokinetics and pharmacodynamics of valproic acid (VPA) in the rat brain. Specifically, we wished to study the hypothesis that the pharmacological function of VPA may be related to its capacity to attenuate brain N-acetyl aspartic acid (NAA) levels.

Initial studies were designed to locate brain regions where VPA significantly changed NAA levels. These two compounds were concurrently analyzed by GC/MS methods in five brain areas: cortex, hippocampus, striatum, cerebellum and brain stem. Because the two compounds are grossly different in their organic solvent extractabilities, our methodology development was based on the formation of N-butyl esters of NAA directly in the biological samples to enhance the hydrophobicity to the compound. Comparisons of the results from these in vitro studies show only marginal drug induced reductions of the NAA levels in the VPA treated rats. However, the difference is statistically significant (student t test p < 0.05) when cerebellum data from pentobarbital pretreated rats were compared.

On-line microdialysis CF-FAB MS methods was then applied to compare pharmacokinetics of brain and blood VPA levels after an IV VPA dose of 200 mg/kg. The detection sensitivity of the Finnigan-MAT 90 CF-FAB MS is 500 pg in 0.5 μl injection volume. Figure 1 shows that blood peak levels of VPA is at least 10 times higher than that in the brain. However, the disappearance rate of the drug in the brain is much slower than that in the blood. The figure shows data for about 3 hours, although the experiment was carried out for over 5 hours.

Off-line microdialysis GC/MS methods were then used to further correlate the brain VPA levels with the concurrently measured NAA and other neuroactive amino acids in live animals. Figure 2 shows correlations between the brain extracellular VPA levels and the NAA levels. The time points when VPA and/or pentobarbital were given are marked along the X-axis. Three observations from these results should be noted. 1) Extracellular VPA levels are dose dependent. 2) pentobarbital injection appears to release free VPA into the extracellular fluid. 3) NAA levels in the brain appear to be affected by the presence of VPA in the brain.

In summary, mass spectrometry is an efficient and effective method to follow brain neurochemistry. We have shown it can be used to monitor intravenous and/or intraperitoneal injections of drugs such as VPA and investigate the effect of this drug on other neurotransmitters in the brain. This is highly significant because these changes can be measured in a live animal while the brain is still functional. Future work will apply these procedures to study seizure disorders in humans.

References

Acknowledgements

The authors wish to thank NIH for part of the financial support on this study.

Figure 1

BRAIN VPA VS NAA

microscopy of brain cry

CONCENTRATION (mg/23.4 ul)

Figure 2

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GC/MS AND LC/MS CHARACTERIZATION OF METABOLITES OF N-FORMYLAMPHETAMINE, A POTENT HEPATOTOXIC CONTAMINANT OF ILLEGALLY SYNTHESIZED AMPHETAMINE
Abdul Mutlib, Michael Legallo and Frank Abbott, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z3 and Department of Pathology, U.B.C. Hospital, Vancouver, B.C., Canada, V6T 2B5.

Introduction
Recently we have described the metabolic fate of (±)-N-(1-methyl-3,3-diphenylpropyl)formamidine(1). It was found that an isocyanate derived glutathione and N-acetylcysteine conjugates were excreted in bile and urine, respectively, of rats dosed with this formamide. N-Formylamphetamine, which bears a structural resemblance to N-(1-methyl-3,3-diphenylpropyl)formamidine and is a contaminant in illicitly synthesized amphetamine, was studied with regard to its disposition in rats. The water-soluble metabolites excreted in urine and bile of rats dosed with N-Formylamphetamine were analyzed by LC/MS/MS. The non-conjugated metabolites were characterized by GC/MS. Furthermore the hepatotoxicity induced in Sprague-Dawley rats after an acute dose of N-formylamphetamine was investigated.

Experimental
Materials: The glutathione and N-acetylcysteine conjugates were synthesized by reacting N-(1-methyl-2-phenylethyl)-4-nitrophenylcarbamate with glutathione and N-acetylcysteine in basic methanol. The non-conjugated metabolic standards were synthesized by the method of Beckett and Morgan(2).

Methods. Animal Experiments. Male Sprague-Dawley rats (250-300 g) were anesthetized with an i.p. injection of urethane (1 g/kg) and bile collected after dosing with N-formylamphetamine, (60 mg/kg, i.p.). To detect the glutathione adduct in the crude bile, selected ion monitoring (SIM) recording and MS/MS of the protonated molecular ion (MH⁺, m/z 469) was carried out using LC/MS. The metabolites released after enzymatic hydrolysis of bile were extracted with ethyl acetate and analyzed by GC/MS. For urine analyses, rats were injected with the same dose of formamide and urine collected for 18 hours. The N-acetylcysteine conjugate in crude urine was detected by LC/MS using SIM recording and MS/MS of the ion (MH⁺, m/z 325). The non-conjugated metabolites in β-glucuronidase hydrolyzed urine samples were extracted with ethyl acetate and analyzed by GC/MS. Formamide induced hepatotoxicity was also investigated in Sprague-Dawley rats (200-300 g) after dosing with N-formylamphetamine and examining processed liver slices (H & E) under light microscope. LC/MS was carried out with the Sciex Heated Nebulizer LC/MS interface coupled to an API/MS/MS system. MS/MS was carried out using argon as the collision gas. HPLC was done on a reversed phase analytical column (Ultrasphere ODS, 5 um, 250 x 4.6 mm) with the solvent being delivered by a dual syringe pump at a rate of 0.7 ml/min. The mobile phase consisted of a gradient system beginning with 20% acetonitrile in 0.01M ammonium acetate (pH 7) and increasing the percentage of organic modifier to 100 % over 12 minutes. GC/MS was performed on a HP5987A GC/MS system using a cross-linked methylsilicone fused capillary column (HP, 20 m x 0.32 mm i.d.). Column temperature was programmed from 50°C to 280°C at 6°C/min.

Results
LC/MS/MS of the protonated molecular ion of the synthesized glutathione (m/z 469) and N-acetylcysteine (m/z 325) adducts gave fragmentations as shown in figure 1. The glutathione adduct gave characteristic ion fragments at m/z 308, 340 (loss of 129), 179 (cysteinylglycine moiety of glutathione) and 162 (substrate). Rats dosed with N-formylamphetamine excreted the glutathione adduct in bile. LC/MS SIM chromatogram of the pseudomolecular ion (m/z 469) of the metabolite from the crude bile sample gave a peak with identical retention time as the synthetic standard (figure 2). MS/MS of the ion (m/z 469) of the metabolite and the synthetic standard produced identical mass spectra. Similarly the presence of the N-acetylcysteine adduct in urine was confirmed by selected ion monitoring recording of the ion (MH⁺, m/z 325). The identities of the non-conjugated metabolites which included benzylmethylketone, amphetamine, 4-hydroxyamphetamine, N-formyl-4-hydroxyamphetamine, norpseudoephedrine and N-formynorpseudoephedrine, were confirmed by comparing GC/MS retention times and mass spectral fragmentations with those of synthetic standards. N-Formylamphetamine was a potent hepatotoxic agent, producing liver necrosis at a dose of 40 mg/kg or more. The necrosis was evident 2-3 hours after an i.p. dose of the secondary formamide to rats.

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Conclusions

Rats dosed with N-formylamphetamine excreted N-acetylcysteine and glutathione adducts in urine and bile respectively. LC/MS/MS was found to be the method of choice for detecting these polar metabolites in urine and bile without any additional purification steps. N-Formylamphetamine was found to be a potent hepatotoxic agent. Apart from bioactivation to an isocyanate, N-formylamphetamine was metabolized by two other pathways, namely: N-deformylation and aromatic and aliphatic hydroxylation.

Figure 1. MS/MS spectra obtained from the protonated parent ions of the synthetic N-acetylcysteine adduct (MH⁺, m/z 325) (top), and the glutathione conjugate (MH⁺, m/z 469) (bottom).

References

SEPARATION AND ANALYSIS OF MACROLIDE ANTIBIOTICS BY CAPILLARY LC/ESI AND CAPILLARY ZONE ELECTROPHORESIS

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Macrolides are a class of antibiotics produced by *Streptomyces* soil bacteria. Macrolides are widely-used broad-spectrum antimicrobial agents, which are useful against gram-positive bacteria. While erythromycin is, perhaps, the most commonly-prescribed macrolide in the United States, other members of this class have been developed and are commonly used in other countries.

During the course of our studies on the mechanisms of drug resistance, the need has become apparent for a physico-chemical method capable of determining both drug purity, degradation products, and enzymatically-induced structural changes in the macrolide molecule. Because of the possibility of formation of several products, a combination of a mass spectrometric technique with a separation technique would be desirable. Since these compounds cannot be analyzed by El or CI direct probe or GC/MS, a LC/MS method would be desirable.

Positive ion electrospray ionization of macrolide antibiotics yield spectra with abundant protonated molecular ions (Figure 1). Structurally-informative fragment ions, resulting from cleavage between the rings, are also observed (Figure 2), and their relative abundance can be enhanced by increasing the skimmer voltage in the Vestec ESI source. This effect probably occurs via a CID mechanism, and is similar to that observed by Loo, et al. (1) for the Fenn/Whitehouse ESI source.

In this study, two nanoscale separation techniques, capillary zone electrophoresis (CZE) and packed capillary LC columns (nCLC), 75 um i.d. were applied to the separation of a series of ten macrolide antibiotics. Both techniques utilize sub-microliter/min flow rates through the analytical capillary and, therefore, require an electrospray probe which incorporates coaxial sheath flow.

Separation of synthetic mixtures of macrolides can be readily achieved using either CZE (Figure 3) or nCLC (Figure 4), but the higher loading capacity of the nCLC column proves distinctly advantageous where mass spectrometric sensitivities are limiting. On the other hand, CZE peak widths are narrower, detection limits are lower, and analysis times are shorter.

Solvent Consideration in Developing Low ppb LC/MS Confirmation Method for Cloxacillin

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Introduction
In order to insure human food safety, new methods must be developed to monitor veterinary drugs that can enter the food chain. Penicillins are a class of drugs that prove difficult to detect and confirm at the low ppb tolerance set for milk or tissue. The development of a method for confirmation of penicillin in milk or tissue requires careful attention to analytical methodology used for sample preparation and storage. This paper investigates the degradation of cloxacillin in various extraction solvents to find the solvent and storage conditions that best maintain the integrity of the sample.

Experimental
Samples of cloxacillin ranging from 500 ng/ml to 1 mg/ml were stored in amber glass vials for up to 8 weeks at -20°C. Every one or two weeks the samples were analyzed by LC/UV. The sample solutions of cloxacillin were analyzed by thermospray LC/MS at the 4-8 week point in the stability study to identify degradation products formed.

Thermospray LC/MS mass spectra were acquired using a mobile phase of 18% Isopropanol, 2.5% acetic acid in 0.25 M aqueous ammonium acetate solution, at a flow rate of 1 ml/min, on an Ultraspher phenyl 3 μm analytical column, 4.6 x 150 mm (Phenomenex, Torrance, CA, U.S.A.). The thermospray interface (Pinniger MAT, San Jose, CA, U.S.A.) was operated with the source temperature and vaporizer temperature at 300°C and 130°C, respectively. The thermospray interface was coupled to a Finnigan MAT 4800 quadrupole mass spectrometer that was operated in the positive ion detection mode scanning conditions from m/z 150 to 600 daltons in two seconds.

Results and Discussion
Thermospray LC/MS aided in the identification of the degradation products of cloxacillin in various solvents. Cloxacillin (Structure I, Figure 1) stored in water, and aqueous solutions of acetonitrile or 2-propanol exhibited a LC peak with a mass spectrum consisting of an [M+H]+ ion at m/z 410 and several protonated fragment ions at m/z 279, 237 and 196. This mass spectrum is consistent for the penilloic acid (Structure II, Figure 1). Since the β-lactam ring was opened in the formation of the penilloic acid, the ion at m/z 160 (characteristics for β-lactams) was absent.

Cloxacillin stored in aqueous solutions of methanol or ethanol gave rise to a LC peak with a mass spectrum exhibiting (for methanol) an [M+H]+ ion at m/z 468 and protonated fragment ions at m/z 309, 196 and 160. This spectrum is consistent with the formation of the methyl ester of cloxacillin (Structure III, Figure 1). Likewise, (for ethanol) the ethyl ester resulted in a mass spectrum with a [M+H]+ ion at m/z 482 and fragment ions at m/z 323 and 160. It was interesting to note that 2-propanol or aqueous solutions of 2-propanol didn't form a propyl ester, rather cloxacillin in that solvent underwent hydrolysis.

The storage of cloxacillin (100 μg/ml) in various solutions (Figure 2) indicated that methanol resulted in the most rapid degradation of this antibiotic while 25% acetonitrile, 25% ethanol in water solution resulted in the least degradation. After two weeks of storage in 50% methanol in water or 100% methanol, cloxacillin was converted entirely to its methyl ester. A 50% ethanol in water solution resulted in a slow hydrolysis of cloxacillin compared to methanol.

The degradation rate of cloxacillin was dependent on concentration. Lower concentrations of cloxacillin (500 ng/ml) underwent a faster degradation. Degradation was nearly nonexistent at the 1 mg/ml level of antibiotic in each solvent.

Conclusions
This study showed that none of the available solvents was ideal for biological sample preparation or chromatographic procedures for trace determination of penicillins. Cloxacillin stored in various solutions underwent two types of degradation: 1) hydrolysis with decarboxylation in the presence of water, acetonitrile and 2-propanol and 2) alcoholysis when methanol or ethanol was present. Since the methanolysis products form at the faster rate, methanol cannot be used in any step of an analytical procedure. With strict control of analytical procedure, 50% ethanol in water or a mixture of 25% acetonitrile and 25% ethanol in water can be utilized when determining penicillin antibiotics in biological fluids and tissues with minimal losses.

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Figure 1. Chemical structure of: I) cloxacillin, II) cloxacillin penicilloic and III) cloxacillin penicilloic acid methyl/ethyl esters.

Figure 2. Degradation of 100 ppm cloxacillin in different solutions during storage for up to 6-8 weeks at -20°C.
In metabolism studies, traditionally phase II conjugates have been analyzed indirectly as the free drug after enzymatic hydrolysis. Extraction and chromatography of the parent drug and conjugates represent challenges due to the broad range of polarity between the drug and its conjugates. We are using LC/MS to determine Phase II drug conjugates in urine and bile. To date, most of this work has been done using thermospray ionization. More recently, the introduction of electrospray ionization has shown promise for characterizing labile glucuronides and sulfates. To evaluate procedures for recovering glucuronides and sulfates from urine and bile, we used estradiol and its conjugates and tested methodology developed for thermospray or electrospray ionization mass spectrometry.

The following experimental procedure was used to recover and analyze the estradiol conjugates:

1) 100 μL urine or bile was applied to a preconditioned Analytichem C8 cartridge.
2) 50 μL spiking solution of each analyte and 20 mM ammonium acetate buffer pH 4.0 was added to the cartridge to achieve a total volume of 1 mL.
3) The cartridge was washed with 1 mL of 20 mM ammonium acetate buffer pH 4.0.
4) The eluate was collected in 1 mL 20:80 20 mM ammonium acetate buffer pH 4.0: acetonitrile.
5) The eluent was evaporated to dryness (N₂), reconstituted in 90:10 100 mM ammonium acetate buffer pH 4.0: acetonitrile and filtered with a 0.02 micron disc filter prior to analysis.

**HPLC Conditions:**
- Mobile Phase Gradient: 95:5 (2 min.) to 20:80 (25 min.) 100 mM ammonium acetate (pH 4.0) : acetonitrile HPLC Column: 25 cm x 4.6 mm YMC ODS-AQ; 1 cm guard column
- Flow Rate: 1.0 mL/min
- Injection volume: 100 μL
- UV detection at 254 nm

**MS Instrumentation:**
- Finnigan TSQ-70 equipped with a Finnigan thermospray source
- Vaporizer temperature: 75-80°C
- Source temperature: 280°C
- Nermag R1010 equipped with an Analytica of Branford electrospray source

**Thermospray Ionization:**
- In the positive ion mode the ammoniated molecule was observed for E-3G and E-17G with little or no fragmentation. These intense ions were easily observed in extracts of either urine or bile. Loss of SO₃ was observed from the ammoniated molecule of the mixed conjugates, E-3G, 17S and E-3S, 17G. Both sulfate conjugates E-3, 17S and E-3S gave only ions arising from SO₃ loss from the protonated molecule.
- In the negative ion mode the deprotonated molecule of the estradiol glucuronides were observed. Analogous to the positive ion spectra, the mixed sulfate/glucuronide conjugates showed SO₃ loss; however, in their negative ion spectra the loss of 176 (glucuronide) was also seen. The mass spectrum of E-3, 17S showed the (M-H⁻) ion and SO₃ loss.

**Electrospray Ionization:**
- In the positive ion mode only the glucuronides of estradiol gave response. Analogous to the thermospray results a strong ammoniated molecule was seen. Similar spectra were obtained for all conjugates, suggesting that either method would serve to characterize the conjugates. Electrospray spectra of mixed sulfate/glucuronide conjugates gave consistently stronger (M-H⁻) ions; however, no parent ion (either singly or doubly-charged) was seen for the bis-sulfate E-3, 17S.

\[
\begin{align*}
R^1 & = R^2 = H \quad \text{Estradiol} \\
R^1 & = \text{HO-SO}_2 \quad \text{E-3S} \\
R^1 & = \text{HO} \quad \text{E-3, 17S} \\
R^1 & = \text{Glucuronide} \quad \text{E-3G} \\
R^2 & = \text{Glucuronide} \quad \text{E-17G} \\
R^1 & = \text{HO-SO}_2 \quad \text{E-3S, 17G} \\
R^1 & = \text{Glucuronide} \quad \text{E-3G, 17G} \\
\end{align*}
\]
Using LC/MS urine or bile extracts may be profiled for phase II metabolites of estradiol. We are investigating whether this simple recovery procedure will be amenable to other steroids or drugs. Alternative procedures which may employ ion exchange or ion-pairing would be less suitable for thermospray and electrospray ionization. The procedure has the added advantage of retaining the parent compound so that phase I metabolites may likely be recovered using the procedure.

While recovery of analyte in the presence of buffers or salts is often not detrimental to analysis using thermospray LC/MS, ionic strength has a large effect upon electrospray ionization. High concentrations of ammonium acetate buffer (>5 mM) caused discharging in the electrospray source, reducing ion sensitivity. Using packed capillary LC columns we achieved some separation and analysis at lower (1 mM) buffer concentrations from standard solutions.
Separation and Identification of Paralytic Shellfish Poisons in extracts of marine matrices by combined Capillary Electrophoresis-Mass Spectrometry*

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Paralytic Shellfish Poisoning (PSP) is one of the most severe forms of seafood intoxication which results from ingestion of mollusks contaminated with toxic phytoplankton. Neurological symptoms of PSP are associated with marine toxins comprising a basic perhydropurine skeleton. The most toxic compound of this family is saxitoxin (STX) [1].

In view of the unpredictable nature of toxic phytoplankton blooms (often associated with "red tides") and the shellfish intoxication that results from it, constant monitoring of PSP toxins is required for public health protection. Presently the mouse bioassay is used for the majority of toxin-monitoring programs in shellfish [2]; however its narrow dynamic range, its variability of dose/response and its logistic constraints have stimulated the development of alternative methods. Recently, a capillary electrophoresis (CE) method with UV detection has been evaluated in our laboratory for the separation and determination of underivatized PSP toxins [3]. Due to the lack of PSP toxin standards, confirmation of electrophoretic peak identities requires a specific and complementary detection system that can easily be interfaced with CE. A CE-MS interface using ionspray ionization was designed and constructed in this laboratory [3] based on a configuration described by Smith et al. [4]. This system comprises a coaxial capillary arrangement in which both CE and make-up flow columns terminate at the ionspray interface. Using electrokinetic injection of STX and NEO standards, detection limits of 12 and 40 pg respectively were obtained. Different buffer systems were evaluated for their separation efficiency and compatibility with mass spectral analysis. Baseline separation of STX, NEO and GTX toxins was successfully achieved using a Trisma buffer (pH 7.2). An example of such separation is shown in Figure 1 and corresponds to low ng injection of different PSP toxins under full mass scan acquisition (130-410 Da). The same electrophoretic conditions also permitted separation of PSP toxins from extracts of marine matrices. Figure 2 shows results of analysis of sonicated and filtered cells of Alexandrium excavatum, using full-scan CE-MS. These traces indicate other endogenous compounds such as arginine, a biosynthetic precursor of PSP toxins. Extracts of contaminated scallop livers were also analysed by CE-MS and confirmed the presence of STX at low μg/mL level. Although these latter extracts were fairly complex, enhanced selectivity was achieved using multiple reaction monitoring in CE-MS-MS analyses of targeted toxins (STX: 300/204, NEO: 316/220, GTX's: 396/298).

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RIE (m/z: 257, 300, 316, 396)

![Graph showing reconstructed ion electropherogram with peaks for STX, NEO, and GTX1-3.](image)

**Figure 1:** CE-MS analysis of PSP toxins. Reconstructed ion electropherogram (top). Selected mass spectra of electrophoretic peak corresponding to STX and NEO (bottom). 20s electrokinetic inj., Trisma pH 7.2, make-up: 0.2% formic, 8 μL/min, 25 kV.

\[
\begin{align*}
\text{[MH]2}^+ : 150 & \quad \text{[MH]}^+ : 300 \\
\text{[MH]}^+ : 316 & \quad \text{[MH]}^+ : 175
\end{align*}
\]

![Graph showing mass/charge (m/z) values for selected ions.](image)

Figure 2: CE-MS analysis of extract from sonicated cells of A. excavatum (90 mg wet cells/mL). Reconstructed ion electropherogram (m/z 175, 179, 252, 300, 316, 464). 10s electrokinetic inj., Trisma pH 7.2, make-up: 0.2% formic, 8 μL/min, 25 kV.

\[
\begin{align*}
\text{ARG} & \quad \text{m/z: 179} \\
\text{STX} & \quad \text{m/z: 252, 464}
\end{align*}
\]

**References:**

COMPARISON OF THERMOSPRAY AND ELECTROSPRAY FOR THE MS CHARACTERIZATION OF THREE RIFAMYCINS

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INTRODUCTION

Rifamycins are a series of antibiotics which have been found to be useful for the treatment of various mycobacterial infections (1,2). The most widely prescribed member of this group is rifampicin which is used for the treatment of tuberculosis and leprosy (3). Recently, Vekey et al. (4) reported the comparison of multiple ionization techniques for rifapentine, one member of this group of antibiotics; this report included thermospray mass spectrometry (TSMS) data, but not electrospray mass spectrometry (ESMS). The utility of ESMS for the analysis of compounds with a high (>2000) molecular weight has received much attention recently; analysts have been slower to investigate the utility of electrospray for low (<1000) molecular weight compounds. We are currently investigating the utility of ESMS and TSMS for the analysis of rifamycin antibiotics. We have analyzed three compounds of this group, rifampicin (MW 822), rifamycin SV (MW 697), and rifamycin B (MW 755), by both TSMS and ESMS. In addition, we have subjected a mixture of these three compounds to combined high-performance liquid chromatography (HPLC)/TSMS.

EXPERIMENTAL

Samples

Rifampicin, rifamycin SV and rifamycin B were obtained from Sigma Chemical Co. (St. Louis, MO).

Electrospray MS

Each standard was made up to a concentration of 50 ng/μl in 50/50 methanol/water. The ES mass spectra were obtained on a Delsi/Nermag R3010 triple quadrupole mass spectrometer equipped with a Delsi/Nermag ES interface. The samples were pumped into the source at a flow rate of 1 μl/min. The mass spectrometer was scanned from m/z 100 to m/z 1000 at a rate of 4 sec/scan.

Thermospray MS

The TS mass spectra were obtained on a Finnigan MAT TSQ 70 triple quadrupole mass spectrometer equipped with a Finnigan TS interface and source. Analyses were performed in the positive ion, discharge-off and filament-off mode. Combined HPLC/TSMS analyses were performed using an Isco model LC-5000 syringe pump connected to a Rheodyne 7125 injector equipped with a 20 μl loop; the outlet of the injector was connected to a Phase-Sep ODS 5 μm particle size HPLC (15 cm x 4.6 mm ID) column. The total HPLC column eluant flowed into the TS interface of the mass spectrometer. The mobile phase was 60/40 methanol/(0.2 M ammonium acetate in water) pumped at a flow rate of 1.2 ml/min. The mass spectrometer was scanned from m/z 200 to m/z 850 at a rate of 2 sec/scan. Typically, the vaporizer was set to 75 °C, while the block (aero) was set to 220 °C. For analyses of a mixture of all three compounds, a solution containing all three compounds at a concentration of about 0.4 μg/μl each in 50/50 methanol/(0.2 M ammonium acetate in water) was prepared.
RESULTS AND DISCUSSION

Under ESMS conditions, as shown in Figure 1, Rifamycin B had an [MH]$^+$ ion at m/z 756 as the base peak, but also a strong [MNa]$^+$ ion. Rifampicin showed an [MH]$^+$ ion at m/z 823 as the base peak and one fragment ion at m/z 791, which can be attributed to the [MH - CH$_3$OH]$^+$ ion. Rifamycin SV showed an [MNa]$^+$ as the base peak plus a second peak at m/z 744 which is the [M + 2Na - H]$^+$ ion.

Under TSMS conditions each compound provided a strong [MH]$^+$ ion: rifampicin at m/z 823 (base peak); rifamycin SV at m/z 698; rifamycin B (see Figure 2) at m/z 756 (base peak). For rifamycin SV, the base peak at m/z 666 can be attributed to the [MH - CH$_3$OH]$^+$ ion and the fragment ion at m/z 648 can be assigned to the [MH - CH$_3$OH - H$_2$O]$^+$ ion. When a mixture of these three compounds was analyzed by HPLC/TSMS, the result was baseline chromatographic separation of the three compounds as well as good mass spectra for each compound in less than 20 minutes.

CONCLUSIONS

Both ESMS and TSMS provided useful data for these compounds. For two of the three compounds, the TS mass spectra were significantly different from the ES mass spectra. TSMS had the advantage that it could readily be used in the HPLC/TSMS mode which provided a technique for the analysis of a mixture containing these three compounds.

REFERENCES

DETECTION OF DETOMIDINE AND ITS METABOLITES IN EQUINE URINE USING AN ATMOSPHERIC PRESSURE IONIZATION LC/MS

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INTRODUCTION
Detomidine, a potent sedative drug, has been illegally used by some horsemen to calm horses before racing. Detomidine is administered to horses in very low doses (5-20mg/horse). In order to regulate its use in the racing industry, a sensitive method is needed to screen and identify it or its metabolite(s) in equine urine and blood. Little is known about the pharmacokinetics of detomidine in horses. Using GC/MS analysis, Seymour et al. (1) had detected two metabolites, 3-hydroxy-detomidine and 3-carboxylic acid detomidine in equine urine. The purpose of our study is to develop an analytical method which can be used to regulate the pre-race use of detomidine and to study pharmacokinetics of detomidine in horses.

EXPERIMENTAL
Plasma Extraction: Equine plasma (5 mL) was adjusted to pH 10 and extracted with ethyl acetate. The organic layer was back extracted into 0.5 N HCl. The aqueous layer was adjusted to pH 10 and extracted with ethyl acetate. The organic layer was dried, reconstituted and analyzed by LC/MS.

Urine Extraction: For detection of hydroxy-detomidines in equine urine, urine (20mL) was hydrolyzed by beta-glucuronidase and cleaned up by a backwash technique as described in the plasma extraction. For detection of detomidine 3-carboxylic acid, urine (5 mL) was purified by a Xtrack™ DAU column (Worldwide Monitoring; Harsham, PA). The eluate was dried, reconstituted and analyzed by LC/MS.

LC/MS: The chromatographic system consisted of an ABI (Foster City, CA) 140A dual syringe pump equipped with a Brownlee® Spheri-5 cyano column (220X4.6mm). The mobile phase was gradient programmed from 50% acetonitrile and 50% 10mM ammonium acetate to 100% acetonitrile in 7 minutes and the flow rate was 0.75 mL/min. The mass spectrum analyses were performed using a Scienx (Thornhill, Ontario) API III triple quadrupole LC/MS system with a heated nebulizer interface.

RESULTS
A sensitive and simple method using atmospheric pressure ionization LC/MS with selective ion monitoring of daughter ions was developed to detect detomidine and its metabolites in equine blood and urine. Detomidine was detected in equine plasma up to 3 and 6 hours post I.V. administration of 5mg and 20mg of detomidine, respectively. Detomidine 3-carboxylic acid and 3-hydroxy-detomidine were detected in the equine urine which corresponds to the reported GC/MS analysis (1). A new metabolite, 4-hydroxy-detomidine, was isolated and identified in the equine urine. The two hydroxy-detomidines were detected in the beta-glucuronidase hydrolyzed equine urine up to 1 and 2 days post I.V. administration of 5 and 20 mg, respectively. No parent compound was detected in the equine urine. The detection limits of detomidine in equine plasma and hydroxy-detomidines in equine urine were approximately 500 pg/mL.
CONCLUSION

Atmospheric pressure ionization LC/MS with selective ion monitoring of daughter ions provided a sensitive and simple method for detecting detomidine and its metabolites in equine blood and urine. Detomidine was detected in the equine plasma up to 6 hours and hydroxy-detomidines were detected in the equine urine up to 2 days post I.V. administration of 20mg of detomidine. Our method can be used to regulate the use of detomidine in the racing industry.

REFERENCES


ACKNOWLEDGEMENTS

Funds for this work were provided by Race Track Division, Agriculture Canada. The authors would like to thank Farmos Groups Ltd., Turku, Finland for providing 3-hydroxy-detomidine and detomidine 3-carboxylic acid for this work. The copyright is owned by Her Majesty the Queen in the Right of Canada represented by the Minister of Agriculture.
DETECTION OF CORTICOSTEROIDS IN EQUINE URINE USING AN ATMOSPHERIC PRESSURE IONIZATION LC/MS

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INTRODUCTION

Synthetic corticosteroids are anti-inflammatory agents which are prohibited for use in race horses at some race tracks in North America. Some of them are administered in very low doses (5 mg/horse) and are hard to detect and identify in equine urine by other conventional techniques. Eight veterinary products, betamethasone, dexamethasone, flumethasone, isoflupredone, methylprednisolone, prednisolone, prednisone, and triamcinolone acetonide, were used in this study. The purpose of our study is to develop an LC/MS analytical method which can be used to regulate the pre-race use of corticosteroids in horses.

EXPERIMENTAL

Urine Extraction: Urine (50mL) was adsorbed on XAD-2 resin at pH 9.5 and eluted with 50% ethyl acetate in dichloromethane. The eluate was dried and redissolved in dichloromethane. The dichloromethane extract was added into a silica solid extraction column and washed with 20% ethyl acetate in dichloromethane. The sample was then eluted with 60% of ethyl acetate in dichloromethane. The eluate was dried, reconstituted and analyzed by LC/MS.

LC/MS: The chromatographic system consisted of an ABI (Foster City, CA) 140A dual syringe pump equipped with a LiChroCART® (Merck) 100 RP-18 column (125X4mm). The mobile phase was gradient programmed from 20% acetonitrile and 80% 10mM ammonium acetate to 100% acetonitrile in 10 minutes and the flow rate was 0.75 mL/min. The mass spectrum analyses were performed using a Sciex (Thornhill, Ontario) API III triple quadrupole LC/MS system with a heated nebulizer interface.

RESULTS

A sensitive and simple method using atmospheric pressure ionization LC/MS was developed to detect synthetic corticosteroids in equine urine. Synthetic corticosteroids show characteristic ions in both positive ion mode and negative ion mode LC/MS. The M+1 daughter ion mass spectra of corticosteroids obtained from the positive ion mode show complex fragmentation patterns, while the negative mode daughter ion mass spectra (both M+59 and M-1) of corticosteroids show simple patterns of fragmentation. After administering the horses with clinical doses of corticosteroids, all the corticosteroids used in this study were detected in the equine urine. Depending on the pharmacokinetics of individual corticosteroids, they can be detected in the urine samples from several hours (e.g. flumethasone) up to several days (e.g. triamcinolone acetonide) post administration. The detection limits of synthetic corticosteroids in equine urine were approximately 1 ng/mL.

CONCLUSION

A technique was developed to detect corticosteroids in equine urine by atmospheric pressure ionization LC/MS. Both positive ion mode and negative ion mode of LC/MS and LC/MS/MS provide characteristic ions and fragmentations for the detection of corticosteroids. Depending on the pharmacokinetics of individual corticosteroids, they can be detected in the urine samples from several hours up to several days post administration. Our method can be used to regulate the use of synthetic corticosteroids in the racing industry.
Selective ion (m/e 469) monitoring of negative ion LC/MS chromatograms of pre-administered urine extract, 0.5 hours post administered urine extract and 8.5 hours post administered urine extract from the horse I. V. administered with 5 mg of flumethasone (Flucort®).

Selective ion (m/e 493) monitoring of negative ion LC/MS chromatograms of pre-administered urine extract, 6 hours post administered urine extract and 120 hours post administered urine extract from the horse I. M. administered with 48 mg of triamcinolone acetonide (Vetelog®).

ACKNOWLEDGEMENTS
Funds for this work were provided by Race Track Division, Agriculture Canada. The copyright is owned by Her Majesty the Queen in the Right of Canada represented by the Minister of Agriculture.
DETECTION AND CHARACTERIZATION OF GLUTATHIONE CONJUGATES USING IONSPRAY/TANDEM MASS SPECTROMETRY

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The formation of glutathione conjugates of electrophilic xenobiotics or their
metabolites represents an important detoxification pathway. The recognition and
characterization of glutathione conjugates correspondingly provides important
evidence concerning the structures of reactive electrophilic metabolites of
potential toxicological importance. In previous work (1,2) we have developed
procedures based on fast atom bombardment /hybrid tandem mass spectrometry
for the screening of complex biological extracts for the presence of glutathione
conjugates. Thus, precursor and constant neutral loss scanning have been used
to detect fragmentations characteristic of this class of conjugate. More detailed
evidence of structure has been obtained by product ion scanning for precursor
ions identified as significant in the screening steps.

Here we report an extension of this approach to incorporate the
ionspray/ electrospray sample introduction technique together with on-line
chromatographic separation of complex biological extracts. The xenobiotic
studied here, 2-furamide, is a substituted furan with hepatotoxic properties. In
contrast to other substitutedfurans, such as the diuretic furosemide, 2-furamide
exhibits a glutathione threshold of toxicity; that is, its toxic properties become
manifest after depletion of hepatic stores of glutathione. This work forms part of a
broader study of structure/ toxicity relationships amongst substituted furans.

Anesthetized, bile cannulated male Fischer rats were administered a 1:1
mixture of unlabelled and [13C4]-labelled 2-furamide at a total dose of 200 mg/Kg.
Bile was collected over 15-30 min intervals for 2 h. Control bile was collected from
each animal for 30 min prior to administration of the xenobiotic. Bile samples
were rendered 1% by volume in heptfluorobutyric acid (HFBA) and extracted on
reverse-phase (C18) Sep-Paks, eluting with acetonitrile/water (4/1) containing
0.1% HFBA. The dried extracts were redissolved in 50 ul of acetonitrile/water
(1/1). 2 ul aliquots of the final solutions were used for LC-ionspray tandem MS
which employed a C18 microbore column (100 x 1 mm). The mobile phase was
programmed from 100% A (0.1% aqueous trifluoroacetic acid (TFA) to 100% B
(0.1% TFA in acetonitrile) in a linear fashion over 40 min, at a flow rate of 50
ul/min. The chromatographic eluent was directed to the ionspray source of a
Sciex API III triple quadrupole mass spectrometer. Air was used as the
nebulization gas at a pressure of 80 psi. The orifice was held at 60 V and the
potential difference between the orifice and the first quadrupole was 30 V.

Conventional and tandem mass spectra were recorded by scanning in 1 Da
increments, with typical dwell times of 5 ms. For tandem MS analyses, the
collision gas was argon and the collision energy was 70 eV.

The total ion current trace recorded during LC-MS analysis of an extract of
bile from a rat dosed 2-furamide showed a complex series of peaks, none of which
could be readily attributed to metabolites of the xenobiotic. The ion current trace
obtained by LC-MS/MS analysis with repetitive constant neutral loss (of 129 Da,
characteristic of glutathione conjugates (1)) scanning, however, suggested the
presence of at least two glutathione conjugates (Figure 1). Inspection of the
constant neutral loss scan corresponding to peak B indicated a precursor ion of
m/z 417/418 (corresponding to unlabelled and [13C4]-labelled species); product
ion spectra for these precursors confirmed the identification of the previously
recognized (2) glutathione conjugate of intact 2-furamide.

The constant neutral loss scan corresponding to HPLC peak A included a
prominent doublet with (1/1 relative abundance) of m/z 420/421. The product ion
spectrum of m/z 420 (Figure 2) substantiated the assignment as a glutathione
conjugate and indicated the mass of the conjugated species to be 113 Da,
corresponding to a probable elemental composition of C₅H₄O₂ (Figure 3).
Additional work is in progress to assign structure.
The data reported here indicate the value of the LC-ionspray/tandem MS combination in xenobiotic metabolism studies. In particular, the combination of chromatographic separation and tandem MS screening has facilitated the recognition of a second glutathione conjugate metabolite of the xenobiotic, 2-furamid.

References

Figure 1. Ion current trace obtained by LC-tandem MS analysis of a rat bile extract with repetitive constant neutral loss scanning of 129 Da.

Figure 2. Product ion spectrum obtained by collisional activation of m/z 420; spectrum recorded during elution of peak A (Figure 1).
Zofenopril calcium (Z) is the S-benzoyl ester prodrug of an angiotensin converting enzyme (ACE) inhibitor SQ-26333 [(S),4-S]-1-(3-mercapto-2-methyl-1-oxopropyl)-4-(phenylthio)-L-proline], (SQ), and is an orally effective antihypertensive agent in man (Figure 1). Urine samples were obtained from normal subjects after administration of single doses of Z or $^{14}$C-Z and the arginine salt of $^{14}$C-SQ. N-ethylmaleimide (NEM) was added to all samples to prevent oxidative degradation of SQ and other metabolites with free thiol groups by formation of NEM adducts. Extensive purification via both preparative and analytical chromatography resulted in the isolation of more than 20 different metabolites.

Mass spectral analysis included chemical ionization and MS/MS analysis on a Finnigan 4600 TSQ, as well as accurate mass FAB and MIKES analysis on a VG-ZAB-HF sector instrument. The formation of methylated derivatives proved extremely useful in the application of accurate mass measurement. Methylation of the carboxyl group provided 1) evidence as to the number of free acid groups in the native metabolite; 2) increased signal intensity for the (M+H)$^+$ ion due to the increased surface activity of the molecule. The application of both high and low energy MS/MS provided complementary fragment ion data, while the use of both positive and negative MS/MS provided structural information on different parts of the molecule due to the charge-directing nature of the fragment ions.

Isolates 8B and 8C were assigned as the diastereomeric pair of NEM derivatives of SQ. Major fragment ions produced in the positive daughter ion MS/MS spectrum are shown in Figure 2. Daughter ions include the m/z 224 fragment ions formed by cleavage of the amide bond with charge retention on the S-phenyl proline moiety, while charge retention on the acylium ion results in the ion at m/z 228. Cleavage on either side of the sulfur atom leads to m/z 324 and 294. Low-energy MS/MS leads to formation of fragment ions at m/z 200 and 158.

The positive and negative FAB mass spectra of metabolites 8A indicated a molecular weight of 339. The fragmentation of the (M+H)$^+$ ion yielded diagnostic ions at m/z 117, 89, and 69, indicating S-methylation (Figure 3). A shift of 16 amu in the 117 fragment ion to 133 was the diagnostic ion in the collisional activation spectrum of the oxidation product.

Mass spectral characterization of metabolite 4C led to its assignment as a novel cyclic thioester. Positive and negative FAB indicated a molecular weight of 485. MIKES produced a neutral loss of 176, indicating a glucuronide conjugate. Accurate mass measurement of the (M+H)$^+$ ion indicated a formula one oxygen less than that expected for SQ-glucuronide. The daughter ion spectrum (Figure 4) of the aglycone ion produced via DCI yielded a fragment at m/z 208, instead of 224 that is usually expected for the S-phenyl proline moiety. The data suggests formation of a seven-membered thioester, which undergoes subsequent reduction and glucuronidation.

CONCLUSIONS: Intact prodrug (zofenopril) was not found in the urine samples after oral doses of zofenopril. SQ, as the NEM adduct, was the principal urinary component. Extensive metabolism included: conjugation with glucuronic acid; conjugation with cysteine; cystathionase cleavage of the cysteine conjugate to the persulfide followed by conjugation with glucuronic acid; S-methylation and subsequent oxidation; oxidation of the S-phenyl moiety; and the formation of a seven-membered thioester glucuronide. Proposed biotransformation of SQ is shown in Figure 5.
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**FIGURE 1**

![Zofenopril Calcium](image1)

**FIGURE 2**

![SQ-36333](image2)

**FIGURE 3**

FRAGMENTATION OF METABOLITE 8A

**FIGURE 4**

FRAGMENTATION OF THE AGLYCONE ION OF METABOLITE 4C

**FIGURE 5**

BIOTRANSFORMATION OF ZOFENOPRIL CALCIUM

- Sulfoxidation
- Enzymatic Hydrolysis
- Reduction and Cyclization
- Methylatton
- Conjugation
- Cystathionase Cleavage and Conjugation

![Biotransformation Diagram](image5)
Biotransformation to polar metabolites followed by conjugation is a typical metabolic pathway of xenobiotics. The chemical properties of the conjugates complicate their analytical characterization and HPLC is often required for direct observation. Thermospray ionization has been used to obtain mass spectral information on conjugates\(^1\). Thermospray is, however, compound specific, requiring several micrograms for the characterization of most molecules. The more recently introduced atmospheric pressure ionization mass spectrometry (API MS) provides and supports two distinct soft ionization approaches for on-line HPLC/MS analysis. In this study, API mass spectra were obtained for glucuronide, glycine, glutathione and sulfate conjugates using heated nebulizer and ionspray API interfaces.

Model conjugates p-nitro-phenyl glucuronide, phenyl glucuronide, dansyl glycine, p-nitro-phenyl sulfate and S-(p-nitro-benzyl) glutathione were dissolved in 50/50 methanol/water.

All mass spectral data were obtained on a SCIEX API III triple quadrupole mass spectrometer equipped with either a heated nebulizer or ionspray API interface. Samples were introduced using flow injection; flow rates were 1 mL/min for the heated nebulizer and 20 μL/min for the ionspray interface. Collision induced dissociation of the conjugates was carried out using argon as the collision gas and collision energies of 10-60 eV. Granddaughter, or ms/ms/ms, spectra were obtained by increasing the declustering energy from 4 eV to 100 eV.

The heated nebulizer inlet allows liquid flows of up to 1 mL/min and produces ions by chemical ionization processes. Spectra obtained using the heated nebulizer inlet consisted mostly of positive or negative pseudo-molecular ions. The only conjugate that produced positive ions in this mode was dansyl glycine. Its spectrum contains an (M+H)\(^+\) at m/z 309 and ions at m/z 251 and 172 from loss of acetic acid and glycine respectively. The last two ions are most likely due to thermal breakdown during the nebulization process. Strong pseudo-molecular anions were observed for glucuronide and glycine conjugates. Figure 1 illustrates the strong (M-H)\(^-\) for phenyl glucuronide. No useful heated nebulizer spectra were obtained for sulfate and glutathione conjugates.

Ionspray reportedly produces ions by an ion evaporation mechanism\(^2\). Strong (M-H)\(^-\) ions were observed for all model conjugates using this mode. The ionspray spectrum for dansyl glycine (Figure 2) is dominated by the (M-H)\(^-\).

Ionspray allowed pg detection limits for sulfate conjugates. It is compatible with mobile phase flow rates below 200 μL/min and would require splitting the flow from standard bore columns. This disadvantage may be offset by superior sensitivity and lack of thermal degradation.

CID daughter spectra were obtained on the pseudo-molecular anions produced by ionspray ionization. The dominant loss for all compounds was the conjugating moiety, as illustrated by the daughter spectrum of p-nitro-phenyl glucuronide (Figure 3). If the instrument is adjusted such that the conjugate fragmented in the declustering region, granddaughter spectra can be obtained. Structurally significant ions, such as loss of NO and NO\(_2\) from p-nitro-phenyl aglycone are readily observed.
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FIGURE 1. Heated nebulizer API negative ion spectrum of phenyl glucuronide.

FIGURE 2. Ionspray API negative ion spectrum of dansyl glycine.

FIGURE 3. CID negative daughter spectrum of m/z 314 from ionspray of p-nitrophenyl glucuronide.
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SIMULTANEOUS DETERMINATION OF TENIDAP AND ITS STABLE ISOTOPE ANALOGUE IN PLASMA BY HPLC/APIMS/MS

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The utility of stable isotope labelled drug to investigate dose\(^1\) or time dependent\(^2\) changes in drug pharmacokinetics; to assess bioequivalence\(^3\) and to demonstrate saturable first-pass effects\(^4\) has long been recognized. Such studies require mass spectrometric assays for the simultaneous determination of the labelled and unlabelled drug.

Tenidap is a new anti-inflammatory agent undergoing clinical evaluation\(^5\). A GC/MS assay for tenidap was not possible as the drug undergoes thermal decomposition in the GC injection port. The limit of detection by thermospray HPLC/MS (2 µg) did not permit the determination of therapeutic levels. HPLC with atmospheric pressure chemical ionization detection proved applicable to the quantitative determination of tenidap in plasma.

Plasma (0.5 mL) was mixed with the internal standard and with 400 µL of 0.05M sodium carbonate and then extracted with 5 mL of ethyl acetate. Following centrifugation, two mL of the organic layer was evaporated to dryness in a vortex evaporator. The dried residue was reconstituted in HPLC mobile phase and 50 µL aliquots were injected onto a CIS column (3.9x150mm). Under isocratic conditions using a mobile phase of 60/40 water/methanol containing 2mM triethylamine at 1 mL/min, the retention times of labelled and unlabelled drug and internal standard (IS) were 2.1, 2.1 and 1.4 min., respectively.

The analysis was performed on a SCIEX API III HPLC/MS/MS system. The effluent from the HPLC column flowed into the atmospheric pressure ionization source via a heated nebulizer interface where chemical ionization was effected by a Corona discharge. Negative ions formed in the source were sampled into the quadrupole mass filter via a 0.0045" sampling orifice. The analysis utilized the technique of multiple reaction monitoring (MRM); i.e. monitoring selected daughter ions of specific parent ions. The collision induced dissociation daughter spectrum of tenidap is shown in Figure 1. The loss of the carboxamide from tenidap, deuterium labelled tenidap and IS parent ions were monitored. The site of deuterium labelling, the thiophene ring, did not affect the measurement. To avoid interference from the naturally occurring D\(_0\)-tenidap isotopes, the analysis of deuterium labelled tenidap utilized its \(^{37}\)Cl ion. The reactions monitored were m/z 319 -> 276 for D\(_0\)-tenidap, 324 -> 281 for D\(_2\) tenidap and 303 -> 260 for the IS.

An HPLC chromatogram of a control plasma sample spiked with 0.1 µg/mL of D\(_0\)- and D\(_3\)-tenidap and a structural analogue IS is shown in Figure 2. Separate experiments using 5 µg/mL concentrations of labelled and unlabelled drug showed no cross-interference. High selectivity of the HPLC/MS/MS detection method resulted in a dynamic range of 0.1 to 5.0 µg/mL. The calibration curves for D\(_0\)- and D\(_3\)-tenidap had correlation coefficients of 0.999. The accuracy and precision of the assay were determined from fortified plasma samples. The assay parameters are summarized in Table 1.

An application of the assay is shown in Figure 3. Plasma samples from rats dosed with equimolar amounts of D\(_0\)- and D\(_3\)-tenidap intravenously were assayed by the present method and a previously developed HPLC/UV method. Samples assayed higher than 5 µg/mL were reassayed after dilution. Total tenidap concentrations for both methods were in excellent agreement.

The technical assistance of Ms. K. Navetta and Ms. D. Farrell is gratefully acknowledged. We are also grateful to Dr. L. Melvin for supplying the deuterium labelled tenidap.
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Figure 1. CID daughter spectrum of m/z 310 from tenldap.

Figure 2. Extracted reaction profiles for MRM of plasma extracts.

Figure 3. Comparison of total tenldap serum concentrations by HPLC/UV and HPLC/MS/MS assay.

Table 1. Analysis of samples containing known amounts of DO- and D3-tenldap: 9 replicates at each concentration.

<table>
<thead>
<tr>
<th>Plasma conc. (µg/mL)</th>
<th>µg/mL calculated ± RSD</th>
<th>DO-tenldap</th>
<th>D3-tenldap</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>0.13 ± 3</td>
<td>0.14 ± 5</td>
<td></td>
</tr>
<tr>
<td>0.20</td>
<td>0.28 ± 4</td>
<td>0.28 ± 7</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>0.46 ± 6</td>
<td>0.48 ± 8</td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>0.88 ± 8</td>
<td>0.89 ± 9</td>
<td></td>
</tr>
<tr>
<td>2.50</td>
<td>2.55 ± 7</td>
<td>2.55 ± 7</td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>4.95 ± 4</td>
<td>4.95 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

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Rapid LC/MS/MS Screening for the Metabolites of Buspirone

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In recent years rapid and comprehensive protocols for drug metabolite identification have evolved with advances in mass spectrometric instrumentation, such as tandem mass spectrometry (MS/MS), HPLC/mass spectrometry (LC/MS) and soft ionization methods (e.g., electrospray). A protocol for metabolite structure elucidation utilizing the advantages of these techniques consists of the following steps:

1. MS/MS screening (1-6) by direct injection of sample into an electrospray source reveals classes of metabolites and preliminary substructural information.
2. LC/MS with gradient elution to observe isobaric metabolites.
3. LC/MS/MS for substructural information on each isobaric metabolite.

A study utilizing this protocol was undertaken to enhance the understanding of buspirone metabolism (6-8) and to study current methodology used in rapid metabolite surveys.

A male Sprague-Dawley rat was administered 10 mg of buspirone HCl by gavage. Bile and urine samples were collected for 0-4 hr. A Sciex API III with an ionspray source was used for all MS analyses in MCA spectral collection mode. For MS/MS screening a 1 microliter aliquot of sample diluted 1:1 with mobile phase B (1:1 acetonitrile:1 mM ammonium acetate, pH 6.8) was injected into the stream of mobile phase B supplied to the ionspray source at 20 microliters/min. A series of MS/MS parent and neutral loss screens, which were specific for substructures of buspirone, were performed with successive injections. LC/MS utilized 5 microliter injections of sample into a Waters Novapak C18 column (150mm X 3.9mm X 5 microns) with gradient elution from 10% acetonitrile:90% buffer to 100% mobile phase B in 20 minutes at 1.0 mL/min. LC/MS/MS analysis was performed under the same conditions while daughter spectra of certain classes of metabolites (observed in MS/MS screening) were acquired.

MS/MS screening by direct injection of bile indicated the presence of parent drug and 13 classes of metabolites (Table 1). These included phase I metabolites, corresponding to addition and dealkylation products, as well as phase II metabolites. These analyses were performed without previous sample preparation, thus saving time and expense with respect to traditional techniques of HPLC fraction collection of radiolabeled peaks. The lack of sample preparation and collection assured minimal sample degradation. Electrospray provided a convenient interface for direct analysis of physiological fluids and a soft ionization technique for labile drug conjugates. Daughter MS/MS spectra provided substructural information on the metabolites. For example, the metabolite class having an addition of an oxygen atom to the parent drug was found to be primarily oxidized on the azaspirone moiety. Another metabolite appears to be a dihydroxy methoxy glucuronide metabolite having addition of OH to the azaspirone moiety and methoxy and glucuronic acid on the pyrimidine ring.

LC/MS gradient analysis with acquisition of full scan mass spectra required only one acquisition of 30 minutes. Isobaric compounds of several metabolite classes from screening were readily separated, as shown in Table 1. Electrospray ionization produced metabolite spectra having abundant (M+H)+ ions with no fragmentation, even for the labile conjugate metabolites. Compounds observed in MS/MS screening indicated which extracted ion current profiles to plot from the LC/MS data. Semi-quantitative estimates of the relative amounts of each metabolite were obtained from the intensity of the extracted ion current profile for each (M+H)+.
Table 1. Metabolites of Buspirone Observed by Direct Ionspray MS/MS Screening and Separated by LC/MS

<table>
<thead>
<tr>
<th>Compounds Observed</th>
<th>Number of Isobars Observed by LC/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(M+H)+ Bile Urine</td>
</tr>
<tr>
<td>Buspirone</td>
<td>386 1 1</td>
</tr>
<tr>
<td>Hydroxy</td>
<td>402 4 3</td>
</tr>
<tr>
<td>Di-hydroxy</td>
<td>418 4 4</td>
</tr>
<tr>
<td>Tri-hydroxy</td>
<td>434 1 2</td>
</tr>
<tr>
<td>Di-hydroxy methoxy</td>
<td>448 1 -</td>
</tr>
<tr>
<td>Des-pyrimidinyl</td>
<td>308 1 -</td>
</tr>
<tr>
<td>Pyrimidinyl piperazine</td>
<td>165 1 -</td>
</tr>
<tr>
<td>Des-pyrimidinyl/piperazine</td>
<td>254 1 -</td>
</tr>
<tr>
<td>Hydroxy pyrimidinyl piperazine</td>
<td>161 - 1</td>
</tr>
<tr>
<td>Hydroxy glucuronide</td>
<td>578 1 -</td>
</tr>
<tr>
<td>Di-hydroxy glucuronide</td>
<td>594 2 -</td>
</tr>
<tr>
<td>Hydroxy methoxy glucuronide</td>
<td>608 2 -</td>
</tr>
<tr>
<td>Di-hydroxy methoxy glucuronide</td>
<td>624 1 -</td>
</tr>
<tr>
<td>Di-hydroxy sulfate</td>
<td>498 1 -</td>
</tr>
</tbody>
</table>

LC/MS/MS analysis of the metabolites was performed with gradient elution while acquiring daughter MS/MS spectra of the (M+H)+ of each class of compounds. This analysis provided detailed substructural information for each isobaric metabolite.

The stepwise protocol for a rapid structural survey of metabolites was applied to buspirone and a wealth of metabolism data was obtained. The same MS/MS instrument and electrospray ion source is used throughout the protocol. Physiological fluids can be directly analyzed. Labile metabolites are readily observed and structurally analyzed. This methodology is routine and is readily applied to similar studies.

References

CHARACTERIZATION OF METABOLITES OF XYLAZINE BY GC/MS AND LC/MS/MS.
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Introduction
Xylazine, 2-(2',6'-dimethylphenylamino)-5,6-dihydro-4H-1,3-thiazine, is a powerful tranquillizer which is commonly used to sedate large animals prior to surgical procedures. It also has the potential of being abused on race tracks due to its calming effects on horses. There are no detailed studies published on the metabolic fate of this compound. Preliminary studies have shown that after administering 35S-labelled xylazine to rats, 70% of the radioactivity was eliminated in the urine with 8% of dose being excreted as unchanged xylazine. The objective of the present study was to define the nature of xylazine metabolites excreted in horse urine. LC/MS/MS using the ScieX APCI/MS/MS system was used to detect and confirm the structures of xylazine metabolites in horse urine and in rat microsomal preparations.

Experimental
Materials: 2-(4'-Hydroxy-2',6'-dimethylphenylamino)-5,6-dihydro-4H-1,3-thiazine and 2-(3'-hydroxy-2',6'-dimethylphenylamino)-5,6-dihydro-4H-1,3-thiazine were produced from xylazine using Fenton's reagent (1). N-(2,6-dimethylphenyl)thiourea was produced by heating 2,6-dimethylaniline with ammonium thiocyanate. 2-(2',6'-Dimethylphenylamino)-4-oxo-5,6-dihydro-1,3-thiazine was synthesized by treating N-(2,6-dimethylphenyl)thiourea with 3-chloropropionyl chloride and cyclizing the product by heating it in the presence of triethylamine at 80°C.

Methods. Animal Experiments. Male Sprague-Dawley rats (250-300 g) were sacrificed and livers removed for microsomal preparation. After microsomal incubation of xylazine, the metabolites were extracted and analyzed by GC/MS and LC/MS/MS. A horse was administered xylazine (1 g) and urine collected over 24 hours. A portion of the urine sample (150 ml) was extracted with ethyl acetate and analyzed by GC/MS. The remaining aqueous phase was passed through an Amberlite XAD-2 column. The metabolites were eluted with methanol and concentrated. The residue, redissolved in sodium acetate buffer (pH 5) was incubated with 8-glucuronidase at 37°C for 18 hours. The aqueous phase was extracted with ethyl acetate and the extract analyzed by TLC and LC/MS/MS. GC/MS was performed on a HP5987A GC/MS system using a cross-linked methylsillcone fused capillary column (HP, 20 m x 0.32 mm i.d). Temperature was programmed from 50°C to 300°C at 10°C/min. LC/MS/MS was carried out with the ScieX Heated Nebulizer LC/MS interface coupled to an API/MS/MS system. MS/MS was carried out using 70 eV collision energy and argon as the collision gas. HPLC was done on a reversed phase analytical column (UltraspHERE ODS, 5 um, 250 x 4.6 mm) with the solvent being delivered by a dual syringe pump (Applied Biosystem) at a rate of 0.7 ml/min. The mobile phase consisted of a gradient system beginning with 20% acetonitrile in 0.01M ammonium acetate buffer (pH 7) and increasing the percentage of organic modifier to 100% over 12 minutes.

Results
A number of metabolites were detected in hydrolyzed horse urine and from the incubation of xylazine with rat liver microsomes (figure 1). The major metabolites identified included the two phenolic compounds 1 and 2. N-(2,6-dimethylphenyl)thiourea, 3 and the thiazine ring oxidized metabolite 4. LC/MS/MS was used to confirm the identities of these metabolites. For example metabolite 4 was identified by LC/MS/MS as shown in figures 2a and 2b.

Conclusions
Metabolites of xylazine were found to be hydroxylated at both the para and meta positions of the aromatic ring. While para ring hydroxylation is a well known metabolic reaction, oxidation at meta position has been reported for only a limited number of compounds (2). These phenolic metabolites could not be successfully analyzed by GC/MS even after derivatization with silylating agents. LC/MS/MS was the method of choice for analyzing complex mixtures such as horse urine for small quantities of metabolites.

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Figure 1. Proposed metabolic pathways of xylazine to give metabolites 1-4. The metabolite shown in brackets was not isolated.

Figure 2a. LC/MS selected ion monitoring chromatogram (top) and CAD spectra of the protonated parent ion (MH⁺, m/z 235) of the synthesized standard, 2-(2',6'-dimethylphenylamino)-4-oxo-5,8-dihydro-1,3-thiazine, 4.
Tandem Mass Spectrometry for the Structure Elucidation of Flavones

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A systematic method for the identification of flavone structures with tandem mass spectrometry (MS/MS) has been developed. During the screening of fermentation broths for inhibition of a particular type of enzyme activity, it was realized that natural products with the flavone chemotype could potentially interfere because of the antioxidant activity of this class of compounds. In order to eliminate fermentation broths that contained this chemotype from further scale up and isolation studies, it was necessary to develop a strategy to quickly identify this class of compound. In previous work (1) we had demonstrated that HPLC-diode array bioassay provide a useful tool for this type of quick identification, and we had shown in preliminary results that mass spectrometry could provide additional information that would allow us to confirm identification based on UV-visible spectroscopy. These results extend these preliminary findings by continuing the utility of LC/MS for molecular weight determination, but also extend the usefulness of the procedure through the use of DCI/MS/MS and LC/MS/MS techniques for structure elucidation and identification.

Here we describe an approach which utilizes the desorption chemical ionization (DCI) and thermospray ionization (TS) modes for the rapid analysis of partially purified broth extracts. DCI/MS/MS techniques are utilized to rapidly propose possible structures while LC/MS/MS techniques are utilized to further elucidate and confirm the flavone structures. The method is based on the fact that flavone structures undergo similar collisionally activated dissociations (CAD) corresponding to cleavage through the benzopyrone substructure. The resulting daughter MS/MS spectra contain a complementary pair of fragment ions indicative of possible structures. A direct comparison, both chromatographic and spectral, to authentic standards is used to confirm the postulated structures.

Full scan DCI mass spectra of standard flavones contain an abundant protonated molecular ion [M + H]+ with very little fragmentation. Thus, an initial molecular weight and purity assessment of the extract can be performed using the DCI mode. The full scan DCI mass spectra of broth extracts contain ions indicative of the molecular weights of possible flavone compounds. The major ions are selected for the corresponding daughter MS/MS experiment to determine if flavone structures are present.

The daughter DCI/MS/MS results obtained with various standard flavone compounds indicate that a common fragmentation pathway exists to generate a pair of complementary fragments. These fragments are structurally significant to flavones and correspond to cleavage through the benzopyrone structure via a retro-Diels-Alder rearrangement. Figure 1 shows the daughter MS/MS spectra of daldzeln and genistelin illustrating this characteristic behavior. The daughter MS/MS spectra of daldzeln contain the complementary benzopyrone fragments at m/z 119 and 137 along with several structurally significant ions at m/z 237, 227, 199, 181 and 91 associated with this retro-Diels-Alder rearrangement. The daughter MS/MS spectrum of genistelin contains the complementary fragments at m/z 119 and 153 with rearrangement ions at m/z 253, 243, 225, 215 and 91.

Daughter MS/MS spectra obtained from the broth extract selecting the major ions observed in the full scan DCI mass spectra contain diagnostic flavone-fragment ion pairs indicative of possible structures (Figure 2). Two flavone structures corresponding to daldzeln and genistelin were proposed directly from the spectra. The daughter MS/MS spectra of the authentic standards (Figure 1) compare well with the compounds contained in the extract (Figure 2) as the same daughter ions were observed with similar relative abundances.

Figure 3 shows the LC/MS chromatogram illustrating the separation of daldzeln and genistelin from a reconstituted broth extract. Separation of the two targeted components is achieved in less than 15 minutes. Full scan background subtracted thermospray mass spectra essentially contain a single ion corresponding to the protonated molecular Ion. Daughter MS/MS spectra obtained for each compound yield similar structurally significant ions as observed with DCI/MS/MS. Also, as demonstrated in the DCI mode, the daughter LC/MS/MS spectra of the broth extract and flavone standards contain essentially the same fragment ions with similar relative abundances.

These results illustrate the utility and versatility of MS/MS techniques for the rapid structure characterization and elucidation of flavone compounds. The DCI mode can be used in conjunction with MS/MS techniques to rapidly determine the presence of major flavone compounds contained in a complex mixture. The utility of the TS mode in conjunction with MS/MS techniques is directed toward the trace analysis of all flavone compounds contained in the mixture. Certainly, a two step approach utilizing both DCI/MS/MS and LC/MS/MS may also be employed.

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Figure 1. The Daughter DCI/MS/MS Spectra of Daidzein and Genistein Illustrating the Characteristic Fragmentation Behavior Associated with the Retro-Diels-Alder Rearrangement.

Figure 2. The Daughter DCI/MS/MS Spectra of Daidzein and Genistein Contained in a Partially Purified Broth Extract.

Figure 3. The LC/MS Mass Chromatograms of Daidzein and Genistein From a Reconstituted Broth Extract. Separation of the Two Targeted Components is Achieved in Less Than 15 Minutes (Scan Time = 1 second).
Differentiation of three regioisomers of hydroxy-3,4-(methylenedioxy)methamphetamine by collisional mass spectrometry using ion trap and triple quadrupole mass spectrometers.

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Recently we reported that 3,4-(methylenedioxy)methamphetamine (MDMA) and its N-demethylated metabolite are hydroxylated at positions 2, 5 and 6 of the 3,4-methylenedioxyphenyl ring in the rat following subcutaneous injection of 20 mg/kg MDMA (as free base) [1]. Regioselectivity was observed for the hydroxylation pathway as indicated by the preferred hydroxylation at position 6 over the other two positions. These regioisomers of either hydroxy-MDMA or its N-demethylated analog gave indistinguishable electron and chemical ionization mass spectra. Initial attempts to differentiate the three regioisomers of hydroxy-MDMA by collisional mass spectrometry using an ITMS failed since collision-induced dissociation (CID) of the protonated molecules (MH+) of the three regioisomers all gave identical daughter ion mass spectra. Similarly, identical daughter ion mass spectra were obtained by CID of the MH+ ions corresponding to the regioisomers of the N-demethylated analog of hydroxy-MDMA. This was unexpected since there is literature report that isomeric compounds can be differentiated by collisional mass spectrometry using an ion trap mass spectrometer (ITMS) [2]. Therefore, this study compares collisional mass spectrometry performed on an ITMS and a triple quadrupole mass spectrometers for differentiation of the three regioisomers of OH-MDMA.

Figure 1 shows the three distinct daughter ion mass spectra obtained by CID of the MH+ ions at m/z 402 using a triple quadrupole mass spectrometer (TQMS). The daughter ion mass spectra were recorded at an attenuation of each parent ion intensity by more than 90% following CID at a translational energy of about 24 eV (E|ab) and with argon as the collision gas. In comparison, CID of the MH+ ions of the three regioisomers using an ITMS all gave identical daughter ion mass spectra; each is characterized by an abundant ion at m/z 275. This suggests that the energy deposition by CID using an ITMS is lower than that obtained with TQMS. Since the structural differences of the three regioisomers are contained in their daughter ions at m/z 275, further CID of these ions using an ITMS were explored for their potential in differentiation of these regioisomers. Indeed, the three regioisomers can be distinguished by further CID of their daughter ions using an ITMS as illustrated by the three different granddaughter ion mass spectra in Figure 2. Comparison of the mass spectra obtained with an ITMS and a TQMS clearly indicate that similar series of ions are present but at different abundances in both mass spectra corresponding to each regioisomer. The overall CID efficiencies of the sequential mass analysis (MS/MS/MS) for 2-, 5- and 6-hydroxy-MDMA were 43, 67 and 41%, respectively, and are much higher than that typically obtained tandem-in-space mass spectrometers. MS/MS/MS analysis of hydrolyzed and derivatized dosed rat liver extract using an ITMS clearly identified 5- and 6-hydroxy-MDMA.

In conclusion, the three hydroxy-MDMA regioisomers can be distinguished by collisional mass spectrometry using ITMS and TQMS. However, MS/MS/MS is necessary for differentiation of the three regioisomers using an ITMS.

Acknowledgement: This work was supported by NIDA Grant 1RO1 DA 05860-01.

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Figure 1. Daughter ion mass spectra of 2-hydroxy-MDMA (A), 5-hydroxy-MDMA (B) and 6-hydroxy-MDMA (C) obtained on a TQMS.

Figure 2. Granddaughter ion mass spectra of 2-hydroxy-MDMA (A), 5-hydroxy-MDMA (B) and 6-hydroxy-MDMA (C) obtained on an ITMS.
DETECTION OF HEROIN AND COCAINE RESIDUES BY
ION MOBILITY SPECTROMETRY

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The President's National Drug Control Strategy calls for the
development of new technologies and the innovative adaptation of existing
technology for use in the war on drugs. As part of an ongoing research
effort the FBI Laboratory has been evaluating a number of new technologies
for use in counternarcotics and counterterrorism investigations. The part
per trillion vapor pressures of heroin or cocaine hidden in packages,
conveyances and on people requires extremely sensitive and selective
detection methods. Therefore, the sampling of particulate residue which
may be on surfaces offers a greater chance of detection.

We have been investigating the application of a commercially
available ion mobility spectrometer (IMS) known as IONSCAN (Barringer
Instruments, Inc., South Plainfield, NJ). Samples are collected on a 0.25
micron teflon membrane filter using a vacuum cleaner. The filter is then
thermally desorbed into the IMS. Sample collection requires less than 30
seconds and the analysis is completed in 3 seconds.

Figure 1 shows a block diagram of the IMS. A calibration compound,
nicotinamide, is admitted automatically with the sample. This serves as
an internal reference to which the mobility of the compounds of interest
are referenced. An audible and visual alarm, controlled by the
microprocessor, indicates the presence of a drug falling within a set of
user defined parameters such as mobility, full width at half maximum, and
intensity. The IMS has been shown to be stable over several weeks without
the need to adjust the alarm parameters. A portable PC is also used to
capture and store the plasmagrams for later review.

Table 1 shows the reduced mobilities for several of the common drugs
of abuse. Laboratory testing has determined the detection limits for
cocaine and heroin to be 5 ng and 15 ng, respectively. Nanogram limits of
detection are observed for a number of other drugs. As shown in Figure 2,
heroin (diacetylmorphine), O-6 monoacetylmorphine and morphine show
predictable behavior with successive losses of the acetate moiety.

Typical law enforcement and forensic applications include gathering
evidence for probable cause, search and seizure, forfeiture
investigations, and locating hidden narcotics. Sampling procedures for
examining vehicles, packages and people for hidden narcotics have been
developed. Typical screening applications include suspects under arrest,
prison inmates or their visitors. Cocaine residue can be detected on the
hands for at least 1.5 hours after brief contact with the drug. Simply
washing the hands failed to completely remove the drug residue.

The IMS can provide a quick screening of evidence. For example,
narcotics residue has been detected on the bookkeeping records of drug
dealers submitted to the FBI Laboratory for document examination. The
size and portability of the IMS can provide on the spot detection of
illegal drugs thus improving the law enforcement officer's efficiency.
Property obtained with proceeds from trafficking in illegal narcotics can
be seized and forfeited in civil proceedings. For example, cocaine
residue was found in the passenger compartment of a helicopter seized by
federal agents during an investigation. Cocaine residue has been detected
in automobiles following canine alert. In a number of cases, duplicate
samples were collected and confirmed by tandem mass spectrometry using a
Finnigan TSQ 70 tandem mass spectrometer.

This is a publication of the Laboratory Division of the Federal Bureau of
Investigation. The use of names of commercial manufacturers does not
constitute or imply endorsement, recommendation or favoring.
Figure 1. Block diagram of the IONSCAN ion mobility spectrometer.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>( K_{o} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heroin</td>
<td>1.040</td>
</tr>
<tr>
<td>O-6 MAM</td>
<td>1.129</td>
</tr>
<tr>
<td>Cocaine</td>
<td>1.160</td>
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<tr>
<td>THC</td>
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<td>Methamphetamine</td>
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</tr>
<tr>
<td>LSD-1</td>
<td>2.385</td>
</tr>
</tbody>
</table>

Table 1. Reduced Mobility for some common drugs of abuse.

Figure 2. Plasmagrams for Heroin, O-6 Monoacetylmorphine, and morphine.
LSD, a popular psychoactive drug of the 1960's, continues today to be a significant drug of abuse. LSD is extremely potent (typical dosages of 40-120 ug) and undergoes rapid and extensive metabolism so that only about 1% of the parent drug is excreted in urine.\textsuperscript{1} Several metabolites have been previously identified,\textsuperscript{1} however, the major metabolic products in man have not yet been determined. As a result, analytical methods have been primarily aimed toward identification and quantitation of the parent drug and an N\textsuperscript{6}-demethyl metabolite, Nor-LSD. Due to low dosage and short half life, LSD often occurs at low pg/ml levels in biological fluids and has been a challenging analytical problem for toxicology laboratories and a concern for drug enforcement agencies.

Because of the complex nature of urine, blood, and other biological matrices, extraction and isolation of LSD yields samples with varying degrees of purity. GC/MS analyses often generate mass chromatograms which are complicated by contaminant peaks that inhibit identification of LSD and metabolites, as well as internal standards. In an effort to significantly improve the specificity for LSD and Nor-LSD over existing GC/MS assays,\textsuperscript{2} MS/MS in combination with several ionization methods has been examined. Relative MS/MS sensitivities were compared for CID of molecular ions in positive- and negative-ion modes, with sample introduction by GC and desorption chemical ionization (DCI). Ammonia and methane reagent gases for CI ionization were compared for each method. Underivatized LSD was analyzed by positive-ion DCI. Due to excellent electron-capture properties, trifluoroacetyl (TFA) derivatives of LSD and Nor-LSD were analyzed by GC/negative-ion CI; trimethylsilyl (TMS) derivatives of LSD, Nor-LSD, and Iso-LSD (a diastereomer of LSD) were evaluated using GC/positive-ion CI. Iso-LSD frequently occurs in synthetic preparations and is believed to be biologically inactive.\textsuperscript{3} All data was obtained with a Finnigan TSQ-70 tandem instrument. Optimum transmission and CID efficiency for selected daughter ions was systematically determined for each ionization method. Generally, Ar collision gas pressure corresponded to about a 50% transmission of the incident ion, while low-energy collisions were at 25 to 45 eV (ELAB). For both positive- and negative-ion CI, ammonia reagent gas generated significantly more intense molecular ions compared to methane for LSD, Nor-LSD, and their derivatives.

Positive-ion DCI/MS/MS for underivatized LSD affords excellent sensitivity, in part due to rapid desorption (1-2 sec). Multiple-reaction monitoring (MRM) of four daughter ions can be achieved at the 10-50 pg level, while full scan CID spectra can be obtained with 1.5 ng or less. Analysis by DCI/MS/MS is suitable for qualitative identification, but does not clearly distinguish LSD from Iso-LSD, both of which commonly occur in urine and blood specimens (e.g., Fig. 1).

The negative-ion CI ionization efficiency for the bis-TFA derivative of Nor-LSD is about 10 times greater than for LSD-TFA, and subsequent low-energy CID of M\textsuperscript{-} of Nor-LSD permits quantitation of several daughter ions at a level of 10-50 pg/ml urine. By comparison, CID analysis of LSD-TFA is considerably less sensitive and requires a 0.5-2.0 ng/ml range for quantitation. In addition, an inherent difficulty with the TFA derivative is the near-coelution of LSD and Iso-LSD, which often leads to ambiguities in identification and quantitation.

Positive-ion GC/MS/MS affords excellent specificity and sensitivity for MRM of several daughter ions of MH\textsuperscript{+} of LSD, Iso-LSD, and LAMPA (internal std.) (Fig. 1). An advantage of the TMS derivative is the clear chromatographic separation of LSD and Iso-LSD. Detection limits for m/z 353, 295, and 280 for LSD-TMS are in the 5-20 pg/ml range, while the limit of quantitation is 50-100 pg/ml urine. The principal dissociation pathways of these isomers are the same but relative ion intensities differ significantly (Fig. 1). Eliminations primarily involve the amide and piperidine-ring functionalities, as shown in Figure 2, in which losses of CH\textsubscript{3}NCH\textsubscript{2} (CID of N\textsuperscript{6}-methyl-\textsubscript{d}\textsubscript{2} LSD supports loss of N\textsuperscript{6} methyl group from the piperidine ring), diethylamide, and diethylamine from m/z 353 are observed from MH\textsuperscript{+}. The CID spectrum of MH\textsuperscript{+} of Nor-LSD-TMS shows corresponding losses of CH\textsubscript{3}N-TMS, diethylamine, and diethylamide. Nor-LSD predominantly acquires a mono-TMS derivative and GC/MS/MS sensitivity is slightly less compared to LSD. The GC/MS/MS assay has also been demonstrated for the analysis of LSD in blood with comparable specificity and sensitivity.

In summary, MS/MS provides a high degree of specificity with sufficient sensitivity for analysis of LSD and LSD metabolites at the low pg/ml level in urine and blood. The positive-ion
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GC/MS/MS method markedly eliminates problems associated with interference peaks that often occur by GC/MS and affords a high level of confidence in the identification of LSD and Nor-LSD.

Acknowledgement: This work was supported by the National Institute on Drug Abuse, grant DA07045-01.

References

Figure 1. GC/MS/MS analysis of a urine sample containing 400 pg/ml LSD and 2.0 ng/ml Iso-LSD, TMS derivative.

Figure 2. CID spectrum of MH+ of LSD-TMS.
Glucuronide Conjugates of Carvedilol: Structure Characterization Using Acetylation Derivatives to Determine Positions of Glucuronidation

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Introduction

Conjugation with glucuronic acid is a common route by which many drugs and xenobiotics are metabolized prior to excretion. Several different nucleophilic functional groups, including hydroxyls, phenols, thiols, amines, and carboxylic acids, may be susceptible to conjugation with glucuronic acid. Determining the positions of glucuronidation can be difficult for xenobiotics which contain both amine and hydroxyl groups. Structures of glucuronide conjugates can sometimes be determined using MS/MS, NMR, or inferred from their stability (or instability) under acidic or basic conditions; however, these approaches each have inherent limitations.

An alternative approach for distinguishing between N- and O-linked glucuronides and determining positions of glucuronidation is described in this presentation. The method utilizes selective acetylation of hydroxyls and/or amines with acetic anhydride under different conditions and characterization of the acetylated products by FAB mass spectrometry. Nucleophilic groups, such as amines and hydroxyls, are readily acetylated in non-aqueous solution by acetic anhydride in the presence of a base, such as pyridine. In aqueous solution, the more nucleophilic amine groups are rapidly acetylated by acetic anhydride, but water (which is present in great molar excess relative to the hydroxyl groups on the metabolites) competitively prevents acetylation of the metabolite hydroxyl groups. Amines and hydroxyl moieties of the metabolites which are conjugated to glucuronic acid are blocked and will not be acetylated. Those which are not conjugated are exposed for acetylation, and can be acetylated depending on the conditions used. After completion of the acetylation reaction, a small portion of the reaction mixture is applied directly to the probe of the mass spectrometer and analyzed by FAB mass spectrometry. N- and O-linked glucuronides can be identified based on the number of acetyl groups which are added to the metabolite under aqueous and nonaqueous conditions.

This method has been used to characterize O- and N-linked glucuronide conjugates of carvedilol, including a carbamic acid glucuronide metabolite. Carvedilol contains an aliphatic hydroxyl, an aliphatic amine, and a carbazole amine, which are all potential sites for glucuronidation. The reaction of carvedilol with acetic anhydride in pyridine and aqueous solutions were investigated first to establish the reactivities of different functional groups on the molecule. The results were used as a reference for interpreting the results obtained for the metabolites. Carvedilol, which possesses β-adrenergic receptor antagonism and non-specific vasodilation activities, is currently being developed for treatment of hypertension and angina. It is metabolized extensively in dog and rat via both oxidation and conjugation pathways.

Experimental

Metabolites 2 and 11 were isolated from rat bile by HPLC. Metabolite 10 was also identified in rat and dog bile, but material used in this analysis was prepared from incubation of carvedilol with dog microsomes fortified with UDPGA and purified by HPLC.

Metabolites were acetylated using acetic anhydride in pyridine or aqueous solution as follows. Approximately 10 µg of metabolite was dissolved in 50 µl of pyridine/acetic anhydride (1:1) and reacted 15 min. Acetylation in aqueous solution was accomplished by dissolving approximately 10 µg of metabolite in 50 µl H2O/ethanol/diisopropylethylamine
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(50:50:1), adding 1 ul of acetic anhydride/acetonitrile (1:9), and reacting for 15 min. An aliquot of the reaction mixture was loaded directly onto the probe and the products were analyzed by FAB mass spectrometry using either glycerol or a mixture of dithiothreitol and dithioerythritol (4:1) as a matrix.

Results and Discussion

Acetylation of carvedilol with acetic anhydride in pyridine yielded a diacetylated product with an [M+H]+ at m/z 491 (Fig. 1), 84 u higher than that of carvedilol (m/z 407). When carvedilol was acetylated in aqueous solution, only one acetyl group was added at the aliphatic amine to produce an [M+H]+ at m/z 449 (Fig 2). The carbazole nitrogen was not acetylated in either reaction.

Metabolite 10 was identified as a glucuronide conjugate of carvedilol and showed an [M+H]+ at m/z 593 and an [M-H]+ at m/z 581. Following acetylation in pyridine, M10 showed an [M+H]+ at m/z 793 (Fig. 3), 168 u higher than that of unreacted M10. This product resulted from addition of four acetyl groups to M10. Three acetyl groups added to the hydroxyls of the glucuronyl moiety and one added to the carvedilol portion. To determine whether the aliphatic hydroxyl or amine of carvedilol had been acetylated in pyridine, M10 was reacted with acetic anhydride in aqueous solution. The mass spectrum for this product, in figure 4 showed an [M-H]+ at m/z 623 which indicates the addition of one acetyl group to the aliphatic amine of M10. Thus, the glucuronide moiety of M10 was linked to the aliphatic hydroxyl of carvedilol. NMR data confirmed this assignment.

Three other glucuronide conjugates of carvedilol (M2, M9, and M11) were characterized using this approach. Metabolite 9 was identified as the diastereomer of M10 and showed essentially identical results from acetylation analyses. Metabolite 2 was identified as a carbazole N-linked glucuronide of carvedilol. Five acetyl groups were added to M2 upon acetylation in pyridine. Three acetyl groups added to the glucuronyl moiety and two acetyl groups added to the carvedilol moiety. Metabolite 11 was characterized as a carbamoyl glucuronide conjugate of carvedilol. The acetylation analyses confirmed that metabolism had occurred at the aliphatic amine. This simple, rapid method should be generally applicable to distinguish between N- and O-linked glucuronide conjugates.
Utilization of FAB MS in the Characterization of Amiloride Analog Binding Protein Photoaffinity Probes.

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Introduction

The diuretic Amiloride (Figure 1), has been widely studied with respect to its inhibition of Na\(^+\)/H\(^+\) exchange and the allosteric modulation of the \(\alpha_2\)-adrenergic receptor.\(^1\) Extensive structure activity studies have shown that alkyl substitution of the 5-amino group results in an increase in activity over that of Amiloride.\(^4\) Researchers have also shown that substitution of the guanidine amine results in the loss of activity. This study has focused on the development of some amiloride analogs which possess a photoaffinity moiety that can be easily radiolabeled. Using the potent diuretic drug ethylisopropyl amiloride as a template, several analogs have been developed which are substituted at the 5-amino (R\(_1\)) and the guanidine (R\(_2\)) positions. These analogs will be tested for biological activity and then be used to covalently bind to the Amiloride binding site of the \(\alpha_2\)-adrenergic receptor to aid in the mapping of the binding pocket. Throughout the synthesis of these analogs both positive and negative FAB were used extensively in the characterization of intermediates and final products as well as in the exact mass determination of final products. This proved to be necessary due to the paucity of structural information which was obtained from \(^1\)H and \(^13\)C NMR spectroscopy.

Experimental

Methyl 3-amino-5,6-dichloropyrazine carboxylate was reacted with an appropriate amine to afford the 5-amino substituted methylpyrazine carboxylate. Reaction with guanidine free base resulted in the formation of the 5-amino substituted Amiloride analog. Reaction with N-hydroxysuccinimidyl-4-azidosalicylic acid resulted in the preferential formation of the guanidine (R\(_2\)) substituted analog with a small amount of bis analog with substitution in both the R\(_1\) and R\(_2\) positions. Reaction of N-hydroxysuccinimidyl-4-azidosalicylic acid with the 5-amino substituted methylpyrazine carboxylate followed by addition of guanidine afforded the analog possessing preferential substitution on the R\(_1\) position.

Results and Discussion

Mass spectrometry was carried out on the final products in order to provide confirmation of structure. Since the final products were nonvolatile and too unstable for GC or DCI/MS analysis, other forms of soft ionization were investigated. Electrospray MS gave intense singly charged molecular ions at moderate exit lens energies. At higher exit lens energies the samples gave several protonated fragment ions. The fragmentation pattern did not give an absolute confirmation of structure but rather a pattern common to these types of substituted pyrazines. Positive and negative FAB proved to be the technique which afforded information which allowed the structural identification of the compounds. Figures 1 & 2 show representative positive FAB spectra showing the structural differentiation of two photoaffinity analogs. It can be seen in Figure 2 how FAB was able to determine the structural difference as a result of the characteristic fragment at m/z 327 which resulted from loss of the terminal amine in the 5 position. This gave a definitive differentiation between the two structural isomers shown.
Figure 1. Positive FAB spectra of analog 1 showing major fragments at $m/z$ 315 and 256. Peaks marked by (*) indicate glycerol matrix peaks.

Figure 2. Positive FAB spectra of analog 2 showing major fragments at $m/z$ 327, 315 and 256. Peaks marked by (*) indicate glycerol matrix peaks.

Supported By NIH Toxicology Training Grant ES 00267

Introduction
AC 301,423 (1) is a potent new antiparasitic agent. It has a structure which is closely related to the milbemycins\textsuperscript{1,2} and avermectins\textsuperscript{3} which have a novel mode of action against a broad spectrum of nematode and arthropod parasites of animals.\textsuperscript{4} Common features of these compounds are a fused cyclohexene-tetrahydrofuran ring system, a bicyclic 6,6-membered spiroketal, and a cyclohexene ring fused to the 16-membered macrocyclic lactone ring. It was expected that 1, its analogs, homologs, and metabolites would show similar fragmentation patterns in the El mass spectrum, allowing compound modifications to be identified. An extensive study of the El fragmentation pattern of 1 was undertaken in order to understand its fragmentation pattern so that related compounds could be more readily identified.

Experimental
Structural assignments for these compounds were determined from the mass spectral data generated from a Finnigan-MAT TSQ 4600 triple-stage-quadrupole mass spectrometer. High resolution El/MS and high resolution thermospray/MS were obtained using a Fourier Transform Ion Cyclotron Resonance (FTICR) spectrometer. This provided the elemental compositions for each of the fragment ions. The instrument used was built at Cyanamid and has been described previously.\textsuperscript{5,6}

Results and Discussion
The El spectrum of AC 301,423, which was generated on the quadrupole mass spectrometer, is shown in Figure 2. The identity of many of the ions in this spectrum were determined by FTICR/MS analysis, which was used to generate high resolution El/MS and thermospray/MS data on the fragment ions of AC 301,423. This provided the elemental composition for each of the fragment ions observed. The peak at \textit{m/z} 527 is the loss of CyH\textsubscript{12}O from the molecular ion, which corresponds to the loss of the C-25-oxygen and the C-25 side chain. To confirm the origin of this fragment, two compounds (2,3) similar to AC 301,423 were analyzed. These compounds differ in the side chain on C-25 with R\textsubscript{4} = ethyl for 2 and methyl for 3 instead of isopropyl. In the case of 2, loss of C\textsubscript{6}H\textsubscript{10}O was observed while loss of C\textsubscript{5}H\textsubscript{9}O was observed for 3. This loss is a characteristic fragmentation route for this class of compounds and can be used to identify the composition of the C-25 side chain. It is proposed that tautomerization of the methoxime makes the parent ion susceptible to a retro-Diels-Alder reaction which results in the loss of the C25 side chain. MS/MS experiments showed that the \textit{m/z} 527 fragment ion loses -OCH\textsubscript{3} to form the ion at \textit{m/z} 496. Loss of CO\textsubscript{2} produces the ion at \textit{m/z} 452 which is followed by consecutive losses of H\textsubscript{2}O (\textit{m/z} 434 and \textit{m/z} 416). The \textit{m/z} 496 ion can lose C\textsubscript{6}H\textsubscript{8}O\textsubscript{3} via a retro-Diels-Alder fragmentation pathway to produce the ion observed at \textit{m/z} 388. The structural assignment of the \textit{m/z} 248 ion is based on the elemental composition obtained from the high resolution mass spectrum. Loss of C\textsubscript{6}H\textsubscript{8}O\textsubscript{3} from the parent ion produces the ion observed at \textit{m/z} 511. This fragmentation is unaffected by changes in
the C-25 side chain. When R₂ is ethyl (4) the corresponding ion at m/z 525 is observed showing that this part of the molecule is not involved in the fragmentation. Substitution of the C-4 methyl with an ethyl group (5) increases this fragment loss by 14 resulting in an ion at m/z 511. This evidence supports a McLafferty rearrangement followed by a retro-Diels-Alder reaction. The retro Diels-Alder reaction involving the C-25 side chain occurs giving rise to the ion at m/z 399. MS/MS experiments showed that the m/z 511 fragment ion produced from the molecular ion of AC 301,423 losses H₂O and -OCH₃ to produce the ions observed at m/z 493 and 480, while consecutive losses of H₂O and -OCH₃ produce the ion observed at m/z 462.

Conclusion

The assignments for the El fragment ions of AC 301,423 were based on the high resolution mass spectrum, MS/MS experiments, and by studying the fragmentations of structurally similar compounds. Together, these El fragment ions provide useful information for the structural assignments of homologs and metabolites of AC 301,423.

References

The analysis of human hair for drugs of abuse is a promising alternative to urine drug testing. Hair provides a long-term record, up to many months, of previous drug use, in contrast to urine, which tests positive for only up to a few days for most drugs. Urine remains a better marker for determining whether erratic behavior or an accident might relate to use of a drug, because drugs or their metabolites would probably be in the urine at the time of the incident, but would not show up in the hair for several days. However, in situations in which individuals are tested regularly to ensure that they are drug-free, hair analysis may become the preferred option. The amount of hair required for analyses is small and can be readily taken from the scalp with no embarrassment or significant cosmetic effects.

Our laboratory has been investigating analytical aspects of the analysis of hair for drugs of abuse. Our efforts have concentrated on morphine (heroin metabolite), cocaine, and cocaine metabolites. One of the surprising findings to date is that much more cocaine than its metabolites is found in hair. Because cocaine is readily decomposed at pH > 7, extraction steps must avoid basic conditions. We have found that extractions with 0.1 N HCl at 45 °C for 20 hours efficiently and reproducibly remove the drugs from the hair. Digestion of the hair with proteinase K at a pH of 6.2 and 37° C is also effective for getting the drugs into solution. Once the drug is in solution, the sample can be treated similarly to a urine specimen. We put it through a solid-phase extraction cartridge, convert the drug to a TMS derivative (except cocaine which is not derivatized), and inject (splitless) it into a GC/MS system equipped with a non-polar, fused silica capillary column and operated in the EI mode with selected ion monitoring. Under these conditions, cocaine and morphine levels down to approximately 1 ng per mg of hair can readily be detected for a 10 mg sample of hair.

We have found some improvement in sensitivity with methane positive ion chemical ionization (CI) for cocaine and benzoylecgonine (BE), a cocaine metabolite. Figure 1 shows the responses from a drug user's hair of the (M+H)+ ions for cocaine, BE, and their deuterated analogs as internal standards. These levels are approximately 0.5 ng/mg; thus much lower levels should be detectable.

The ethyl ester of BE has been suggested as a possible cocaine metabolite formed in the liver if alcohol is also present in the blood. Positive ion CI was used to look for this metabolite in the hair of cocaine users. Figure 2 shows the mass spectrum of a peak at the correct retention time for this metabolite. The ions at m/z 318 and 196 correspond to the (M+H)+ ion and the (M+H-C6H5COOH)+ ion, respectively, for the ethyl ester of BE. This metabolite was found in two of five hair samples that were positive for cocaine.

Because the sample preparation for hair analysis by GC/MS is time-consuming, efforts have been made to use direct probe MS/MS for screening hair samples. When we tried this technique on powdered hair samples, the technique worked well. That is, hair with cocaine present was easily distinguished from drug-free hair. However, when short pieces of hair were tried, the results were not as definitive. The technique might be made to work reliably for screening samples, but it is unlikely that it will ever be quantitative.

NIST has conducted two interlaboratory comparison studies involving laboratories interested in the analysis of hair for drugs of abuse. For the first exercise, laboratories were sent eight samples of cryogenically powdered hair for analysis by their methods. The samples included drug-free hair, drug-users' hair, and hair that had been fortified with drugs. Most of the laboratories used acid extractions, with others choosing enzyme digestions that dissolve the hair. GC/MS or a combination of radioimmunoassay (RIA) and GC/MS were the analytical techniques of choice for most laboratories, although there is some work using MS/MS with or without prior extraction. Qualitatively, the results were excellent, with no false positives reported and only one laboratory out of eight missing some of the true positives. Quantitatively, CV's ranged from 15-27%, with results slightly better for morphine than for cocaine. For the second exercise, the laboratories were each sent eight samples of hair cut into short lengths (2-5 mm, typically). Two of the ten laboratories, neither of which used GC/MS, reported some false positives for morphine. There were no false positives for cocaine. Most of the
seven instances of failure to find drugs present also involved morphine. Quantitatively, the results were much more scattered than they had been for the hair powder, a reflection of the greater difficulty in extracting the drug from intact hair segments and possibly the poorer homogeneity of the samples.

One of the most important questions that must be resolved is the differentiation of drugs in hair as a result of drug use from drugs present through environmental contamination. We found that it is very difficult to remove all of the cocaine deposited on hair through vapor deposition. Washes with methylene chloride were the most effective treatments found for removing the vapor-deposited cocaine, but some still remained, even after three 15 min washes. This level of treatment also extracts some cocaine in the hair through drug use. The most promising approaches to resolving this question appear to be finding metabolites that come only from drug use or measuring ratios of drugs found in the hair to drugs found in the wash solutions.

Another environmental aspect involves the effect of various hair treatments on drug levels. We have found that strong solutions of hydrogen peroxide significantly reduced cocaine levels in hair. Other treatments found to reduce the levels, in decreasing order of effectiveness, include permanent waves, shampoos, and salt water. Initial experiments have been with harsher conditions than would normally be applied to hair. Further work will determine the effects of more reasonable treatment regimes.

For the analysis of hair for drugs of abuse to be accepted, laboratories will have to demonstrate a high level of accuracy, both qualitatively and quantitatively. To this end, there is a need for reference materials consisting of human hair with known concentrations of drugs of abuse. NIST is now developing two drugs-in-hair reference materials, one of which will be a powdered hair material and the other will be a short hair segment material. From our own testing and from results in the interlaboratory studies, it appears that hair fortified with drugs, by soaking in dimethylsulfoxide solutions of the drugs, is an acceptable surrogate for drug users' hair. With this material, it is much easier to achieve reasonably homogeneous concentrations than would be the case with drug users' hair where the levels vary considerably within the hair of one individual and even more between individuals.

Figure 1: SIM of cocaine and benzoylecgonine (BE) in human hair using methane positive ion chemical ionization. Drug levels are approximately 0.5 ng/mg of hair. The traces from the top are the \((M+H)^+\) ions for cocaine, cocaine-d\(_3\), BE-TMS, and BE-d\(_3\)-TMS, resp.

Figure 2: The methane positive CI spectrum of the ethyl ester of benzoylecgonine found in the hair of a drug user.
Mass spectrometric analyses of monoamine oxidase inhibitors: Can routine quantitative trace analyses by gas chromatography-mass spectrometry (GC/MS) be done on a LC/MS machine?

*W. Bart Emary, Darlene K. Satonin, Jonathan A. Ragner, G. Douglas Sproles, Marion Merrell Dow Inc., Indianapolis, IN 46268-0470

Instrumental conditions must be optimum to obtain precise and accurate peak ratios for trace level GC/MS analysis of drugs isolated from biological matrices. For GC/MS, the most frequently used sources are either electron impact (EI) or chemical ionization (CI). We have investigated the possible negative effect of introducing semivolatile compounds used for thermospray and FAB on GC/MS instrument performance.

The concentrations of two monoamine oxidase inhibitors (MDL 72,974 and MDL 72,394) in rat and monkey plasma have been measured in separate GC/MS studies. Table I shows typical precision and accuracy results of a quantitative study on the monoamine oxidase B inhibitor, MDL 72,974 in rat plasma. These data were obtained using GC/MS before thermospray or FAB were installed on the instrument.

Table I. Precision and Accuracy Data for MDL 72,974 in Rat Plasma (1.0 mL aliquots)

<table>
<thead>
<tr>
<th>Sample Concentration</th>
<th>Mean Percent Recovery (n=10)</th>
<th>Relative Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDL 72,974</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 ng/mL</td>
<td>104%</td>
<td>4%</td>
</tr>
<tr>
<td>25 ng/mL</td>
<td>102%</td>
<td>4%</td>
</tr>
</tbody>
</table>

![Chemical structures of MDL 72,974, MDL 72,394, and MDL 72,392](image)
Table II shows precision and accuracy data for a quantitative study of a monoamine oxidase type A inhibitor, MDL 72,394 and its metabolite MDL 72,392 in monkey plasma. Standard curves were obtained from 0.5-200 ng/mL in rat plasma and from 2-400 ng/mL in monkey plasma shown below. These data were collected while intermittently using thermospray and FAB for periods of time of up to one week. We have used the two desorption ionization sources for metabolite identification and for work on thermally labile compounds such as peptides and antibiotics. Our results thus far have indicated the performance of the mass spectrometer does not appear to seriously degraded in GC/MS mode after using semivolatile compounds during FAB and thermospray.

Table II. Precision and Accuracy Data for MDL 72,394 and MDL 72,392 in Monkey Plasma (0.25 mL aliquots)

<table>
<thead>
<tr>
<th>Sample Concentration</th>
<th>Mean Percent Recovery (n=10)</th>
<th>Relative Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDL 72,394</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 ng/mL</td>
<td>110%</td>
<td>8%</td>
</tr>
<tr>
<td>40 ng/mL</td>
<td>97%</td>
<td>3%</td>
</tr>
<tr>
<td>400 ng/mL</td>
<td>102%</td>
<td>3%</td>
</tr>
<tr>
<td><strong>MDL 72,392</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 ng/mL</td>
<td>106%</td>
<td>8%</td>
</tr>
<tr>
<td>40 ng/mL</td>
<td>94%</td>
<td>9%</td>
</tr>
<tr>
<td>400 ng/mL</td>
<td>105%</td>
<td>8%</td>
</tr>
</tbody>
</table>

* Sequence run within 2-3 days after thermospray and FAB
SIMULTANEOUS GC/MS ANALYSIS OF LABELED AND UNLABELED NITROGLYCERIN IN HUMAN PLASMA FOR DETERMINATION OF TRANSDERMAL FLUX RATES

M. Leal, J. Joshi, D. Gaudry, A. Pirano, J. Morgan, M. Hayes, and M. Powell

Drug Development Department, Pharmaceuticals Division
Ciba-Geigy Corporation
Ardsley, New York 10502

Nitroglycerin (glycerol trinitrate) is a potent vasodilator used in the treatment of angina. The kinetic behavior of nitroglycerin is such that major fluctuations in plasma levels are produced with changes in posture, physical activity and ingestion of food. Historically, the intrinsic variability in generated nitroglycerin plasma level data made it impossible to produce acceptable statistical power in bioavailability/bioequivalency studies. The simultaneous administration of transdermal nitroglycerin and intravenous \([^{15}N]\)-nitroglycerin should permit bioavailability/bioequivalency to be established with sufficient power in a reasonable number of subjects.

A GC/MS method was developed and validated to simultaneously quantitate plasma levels of unlabeled and stable isotope labeled nitroglycerin. This method utilizes 1,2,4-butanetriol trinitrate as the internal standard. Samples (1 ml) were extracted with pentane/ethyl acetate (90:10). Extracts were concentrated and analyzed directly by GC/MS. Separation was accomplished on a (0.5 u) methyl silicone capillary column (25 m x 0.32 mm I.D.). Mass spectrometry was carried out under negative ion methane Cl conditions with selected ion monitoring of fragment ions at m/z = 62, 62 and 63 for nitroglycerin, 1,2,4-butanetriol trinitrate and \([^{18}N]\)-nitroglycerin, respectively.

Calibration curves were generated using \(Y = AX^8 + C\) as the regression model and were represented by a plot of the amount added versus the measured peak area of labeled or unlabeled nitroglycerin/1,2,4-butanetriol trinitrate. The limit of quantification (LOQ) was 50 pg/ml. Figure 1 shows a chromatogram at the LOQ.

Recovery and reproducibility assessments indicate good accuracy and precision over the validated concentration range of 50 to 1000 pg/ml. Table 1 contains the inter- and intra-day accuracy and precision data for nitroglycerin and \([^{15}N]\)-nitroglycerin respectively, expressed as mean percent found and percent CV. Over the entire concentration range the inter-day precision was between 1.0% and 10.8% for nitroglycerin and between 0.48% and 9.9% for \([^{15}N]\)-nitroglycerin. Although the intra-day precision was <15% in most cases, there were sporadic instances where values up to 22% were noted.

A two-treatment, randomized crossover study was designed to compare the in vivo performance of a new investigational transdermal system with a commercially available system (Transderm Nitro-10\(\text{R}\)) in seven healthy male volunteers. Each subject received one of two dosing sequences, investigational followed by commercial or commercial followed by investigational. \([^{15}N]\)-Nitroglycerin was simultaneously administered as an infusion at a constant and known rate to obtain an estimate of systemic clearance. Both transdermal systems exhibited plasma profiles with significant fluctuations in plasma concentrations. Since these fluctuations were also noted in the plasma profile of the stable isotope infusion, this suggests that the random fluctuations observed reflect variations in clearance with time rather than changes in delivery from the transdermal system. Figure 2 is a representative drug profile from a typical subject given one of the unlabeled transdermal systems and a simultaneous stable isotope labeled infusion.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Table 1
Inter- and Intra-Day Accuracy and Precision Data for Nitroglycerin and $[^{15}\text{N}]-\text{Nitroglycerin}$ Quality Control Samples

<table>
<thead>
<tr>
<th>Added Concentration (pg/ml)</th>
<th>Nitroglycerin</th>
<th>Mean Relative Recovery (CV %)*</th>
<th>Mean Inter-Day Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
</tr>
<tr>
<td>50</td>
<td>108.9 (19.1)</td>
<td>102.7 (7.5)</td>
<td>102.2 (7.3)</td>
</tr>
<tr>
<td>200</td>
<td>86.1 (5.6)</td>
<td>106.7 (13.6)</td>
<td>100.5 (2.0)</td>
</tr>
<tr>
<td>800</td>
<td>85.9 (3.1)</td>
<td>92.7 (7.2)</td>
<td>100.1 (3.7)</td>
</tr>
<tr>
<td>1000</td>
<td>104.2 (20.0)</td>
<td>104.3 (19.4)</td>
<td>102.4 (1.2)</td>
</tr>
</tbody>
</table>

Overall Mean Recovery: 100.3%

<table>
<thead>
<tr>
<th>Added Concentration (pg/ml)</th>
<th>$[^{15}\text{N}]-\text{Nitroglycerin}$</th>
<th>Mean Relative Recovery (CV %)*</th>
<th>Mean Inter-Day Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
</tr>
<tr>
<td>50</td>
<td>96.9 (16.7)</td>
<td>102.2 (8.0)</td>
<td>89.5 (19.5)</td>
</tr>
<tr>
<td>200</td>
<td>88.3 (12.5)</td>
<td>104.7 (12.4)</td>
<td>99.7 (2.0)</td>
</tr>
<tr>
<td>800</td>
<td>84.7 (5.0)</td>
<td>91.9 (8.0)</td>
<td>103.2 (6.0)</td>
</tr>
<tr>
<td>1000</td>
<td>103.8 (81.8)</td>
<td>104.4 (15.9)</td>
<td>103.5 (2.3)</td>
</tr>
</tbody>
</table>

Overall Mean Recovery: 97.6%

*N = 4 for intra-day; N = 12 for inter-day

Figure 1
Selected ion chromatograms for control plasma samples spiked with 50 pg/ml nitroglycerin and $[^{15}\text{N}]-\text{Nitroglycerin}$. Spiked samples contain internal standard (1,2,4-butanetriol trinitrate) at 1.22 ng/ml.

Figure 2
Plasma Concentration of Nitroglycerin and $[^{15}\text{N}]-\text{Nitroglycerin}$

632
APPLICATION OF AUTOMATED GC/MS FOR THE QUANTITATIVE ANALYSIS
OF CGS 18102A IN PLASMA

M. Leal, M. Hayes, H. Lau and M. Powell
Development Department, Pharmaceutical Division,
CIBA-GEIGY Corp., Ardsley NY 10502

CGS 18102A, Figure 1, a novel hexahydrobenzopyridine, has a mixed pharmacological profile of 5-HT-1A agonist and 5-HT-2 antagonistic properties. Based upon these mechanisms, the compound is predicted to have anxiolytic efficacy with possible efficacy in depression.

An analytical method for the determination of CGS 18102A in human plasma has been developed and validated. The method is based on gas chromatography/mass spectrometry (GC/MS) and utilizes CGS 18416A (Figure 1) as the internal standard. Samples (1 ml) were extracted with pentane/ethyl acetate (75:25). Extracts were then concentrated and analyzed directly by GC/MS. Separation was accomplished on a (1 u) methyl silicone capillary column (30 m x 0.32 mm I.D.). GC/MS was carried out under positive ion ammonia CI conditions, with selected ion monitoring of the [M+H]⁺ ions (m/z = 262 and 248) for CGS 18102A and CGS 18416A, respectively.

Calibration curves were prepared in duplicate at the following concentrations: 0.2, 0.5, 1, 5 and 20 ng/ml. Calibration curves were generated using weighted linear regression of the measured peak area ratios of CGS 18102A/CGS 18416A versus concentration. A summary of the calibration curve data is shown in Table 1. An average correlation coefficient of 0.992 was obtained for CGS 18102A, indicating a good fit to the weighted linear regression model. The mean slope data had a coefficient of variation of 7.7% for CGS 18102A.
Table 1
Reproducibility of CGS 18102A Daily Calibration Curves
(0.2 - 20 ng/ml)

<table>
<thead>
<tr>
<th>Analysis Day</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation Coefficient</th>
<th>Mean Error*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9321</td>
<td>-0.0423</td>
<td>0.9921</td>
<td>7.06</td>
</tr>
<tr>
<td>2</td>
<td>0.9482</td>
<td>-0.0117</td>
<td>0.9930</td>
<td>7.22</td>
</tr>
<tr>
<td>3</td>
<td>0.8214</td>
<td>-0.0567</td>
<td>0.9914</td>
<td>9.26</td>
</tr>
<tr>
<td>Mean S.D.</td>
<td>0.901</td>
<td>-0.037</td>
<td>0.992</td>
<td></td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>0.069</td>
<td>-0.023</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

* Mean Error - Mean of the absolute deviation of each standard from the calibration curve.

The overall accuracy and precision of the method was determined by analyzing quality control samples (prepared in advance and stored frozen) spiked with CGS 18102A at 0.2, 0.8, 2 and 20 ng/ml in replicate (N=4) on each analysis day. The overall accuracy was 103.0% for CGS 18102A. Table 2 contains the calculated inter- and intra-day precision for CGS 18102A expressed as mean percent CV.

Table 2
Inter- and Intra-Day Accuracy and Precision Data for CGS 18102A Quality Control Samples

<table>
<thead>
<tr>
<th>Added Concentration (ng/ml)</th>
<th>Mean Relative Recovery (CV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>0.2</td>
<td>110.5 (4.7)</td>
</tr>
<tr>
<td>0.8</td>
<td>100.8 (6.5)</td>
</tr>
<tr>
<td>2.0</td>
<td>100.5 (10.5)</td>
</tr>
<tr>
<td>20</td>
<td>95.0 (5.8)</td>
</tr>
</tbody>
</table>

The method was applied to a single-blind, ascending multidose safety and tolerability study conducted in eight normal healthy male volunteers. Five doses of CGS 18102A were administered (1, 5, 10, 20 and 30 mg) two times a day (b.i.d) for five consecutive days at each dose level (25 days total). At the first four doses, blood for determination of trough plasma levels of CGS 18102A were collected on day 5 at 0 hr and 12 hr. At the highest dose (30 mg) plasma profiles were generated over 24 hrs. The maximum concentration found was 6 ng/ml. Large subject variability was noted with respect to half-life and AUC.
ANALYTICAL METHODOLOGY FOR THE DETERMINATION OF TRICHLOROCARBANILIDE IN HUMAN BLOOD SAMPLES

Robert G. Orth, Jay M. Wendling, Sally A. Walker, Jon R. Wehler, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, Missouri 63167

Trichlorocarbanilide (TCC) is an active ingredient in deodorant soaps. Researchers have shown that small amounts of TCC are absorbed through the skin (1). In humans, TCC is primarily metabolized to N-glucuronides and the sulfate conjugate of 2'-hydroxy-3,4,4'-TCC (2,3). A methodology for the analysis of the latter metabolite has been published (4) but portions of this method are cumbersome and recoveries are poor. The method reported here utilizes similar techniques previously reported (4) but was found to significantly improve recoveries and decrease sample preparation and analysis time by a factor of four. The method involves chemical cleanup of the plasma followed by a two-step derivatization process. The method was required to have detection limits of 1 nanogram per mL of plasma and be highly specific for the analyte. Thus a high resolution gc/ms approach was taken.

The analytical approach involves 3 steps chemical isolation, hydrolysis of the TCC-sulfate followed by derivatization with MSTFA, and analysis on high resolution gc/ms. Isolation of the TCC-sulfate was carried out by spiking the plasma with d6-TCC-sulfate and filtering the plasma. The plasma sample was then added to a 0.5 g octadecylsilane column along with 0.5 mL rinsate of 0.5 M potassium acetate. The column was washed with two three mL portions of 0.5 M potassium acetate followed by 1 mL water. The column is washed with 0.55 mL of 3:1 methanol to water which is followed by 1 mL 50% acetonitrile/water. TCC sulfate is eluted with 1 mL 50% acetonitrile/water and filtered. The acetonitrile/water is removed under a stream of nitrogen at 50 °C. The hydrolysis is accomplished by adding trifluoroacetic acid and heating at 40 °C for 15 minutes. The sample is dried again and derivatized with 0.02 mL of a MSTFA solution containing 1% pyridine and heated at 60-100 °C for 5 minutes.

The gc/ms was obtained using a DB-5 column and a VG-ZABE operated at 36 eV electron impact and a mass resolution >5000. The mass spectrometer was operated in selected ion monitoring. The masses monitored included 548, 546, and 533 for the TMS-TCC and 546 and 556 for d6-TMS-TCC. The criteria for selectivity was based on retention time as compared to the d6-TMS-TCC, the chlorine isotopic ratios, and the presence of the fragment peak at 533.

The method was validated by spiking a pooled plasma sample and determining the standard addition curve for the set of spikes. This standard addition curve was then compared with a standard curve obtained by hydrolysis and derivatizing a set of TCC-sulfate standards. The results are shown in Figure 1. As can be seen there is no matrix effect indicated since the slopes of the two curves are the same within experimental error. The only difference is in the intercept indicating that this pooled sample contains a background level of TCC-sulfate. In addition, to the standard addition comparison a spiking recovery program was completed the results of which are shown in Table 1. All the spikes except one were recovered within the error limits of the measurement. The detection limit was found to be 0.2 ng/mL where as the limit of quantitation which can vary with sample was in general no higher than 1 ng/mL.

References
TABLE 1. RESULTS OF SPIKING STUDY.

POOLED PLASMA SAMPLES SPIKED AT TWO DIFFERENT LEVELS AND ANALYZED.

<table>
<thead>
<tr>
<th>ng/mL ADDED</th>
<th>ANALYSIS RESULTS (ng/mL)</th>
<th>RECOVERY OF SPIKE (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>2.2 ± 0.3</td>
<td>2.2</td>
</tr>
<tr>
<td>2.1</td>
<td>2.9 ± 0.4</td>
<td>2.9</td>
</tr>
<tr>
<td>6.0</td>
<td>9.3 ± 0.7</td>
<td>9.3</td>
</tr>
<tr>
<td>6.0</td>
<td>9.8 ± 0.5</td>
<td>9.8</td>
</tr>
</tbody>
</table>

SECOND PLASMA POOL

| 2.1         | 5.3 ± 0.3                | 5.3                      |
| 2.1         | 5.0 ± 0.4                | 5.0                      |
| 6.0         | 9.0 ± 0.5                | 9.0                      |
| 6.0         | 9.6 ± 0.9                | 9.6                      |

* DETERMINED BY SUBTRACTING NONSPIKED PLASMA RESULTS.
Introduction
Benzodiazepines have been prescribed over the years for their anxiolytic, sedative hypnotic, anticonvulsant and muscle relaxant properties. Since the introduction of chlordiazepoxide in 1961, over 3000 of these compounds have been synthesized and about 35 are in clinical use throughout the world today (1). Although these are relatively safe drugs they can be insidious and must be used with care. High doses for prolonged periods of time can lead to dependence or if combined with alcohol or other CNS depressants can produce coma and death (2-4). Because of the wide availability of these drugs the clinical and forensic toxicology laboratory must be able to screen for, confirm the presence of and quantitate benzodiazepine urinary excretion products.

The urinary benzodiazepines as a group are difficult to identify and quantify. They are extensively metabolized making it sometimes difficult to identify (among the multitude of possibilities) the parent compound ingested. The newer members of this drug class (including alprazolam and triazolam) are therapeutically effective at much lower dosage levels and generally appear in the urine at lower levels as well. Of special interest are the alprazolam samples because of their frequent occurrence and the identification of α-hydroxyalprazolam in human urines by GC/MS is not well documented in the literature.

Materials and Methods

Chemicals
Pure samples of the α-hydroxy metabolites of alprazolam and triazolam were generously supplied by the Upjohn Co. The fluazepam metabolite, 2-hydroxyflurazepam, was generously supplied by Hoffman LaRoche. The remaining benzodiazepine compounds (2H5-oxazepam, oxazepam, temazepam, and lorazepam) and β-glucuronidase were obtained from the Sigma Chemical Co. Derivatives were formed using N-methyl-N-t-butyl-dimethylsilyl trifluoroacetamide (MTBSTFA + 1% BDMCS) which was obtained from the Regis Chem. Co.

Sample Analysis of Unknowns

With each batch run, urine standards (25, 50, 100, 300 and 600 ng/mL), a positive control (200 ng/mL) and a negative control (certified blank urine) were prepared. Unknown specimens (5 mL) were first hydrolyzed by adding 1.0 mL of a 1M sodium carbonate solution. Benzodiazepine compounds were extracted with 10 mL of 20% n-butyl alcohol. The tubes were returned to the autosampler and evaporated to dryness. The control mix was then added to this urine extract matrix, blown to dryness and derivatized as normal.

Enzymatic Hydrolysis, Extraction, and Derivatization Procedure

Complete t-butyldimethylsilyl- derivatization was achieved in the capped centrifuge tubes using 50 μl MTBSTFA and 50 μl of ethyl acetate with heat at 90°C for 60 min. The tubes were then cooled, the autosampler was loaded and 1μl of each sample was injected.

Assay Statistics

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated from the signal to noise ratio (S/N) of the quantitation ion for each analyte. The LOD and LOQ were defined to have a signal to noise ratio of three (S/N = 3) and ten (S/N = 10), respectively. Recoveries (extraction efficiency) were calculated as the ratio of peak area in the extracted control to non-extracted control, each in triplicate, multiplied by 100. The non-extracted control was made up by extracting certified blank urine and blowing the organic to dryness. The control mix was then added to this urine extract matrix, blown to dryness and derivatized as normal.

Sample Analysis of Unknowns

Thirteen urine specimens, either screened positive for benzodiazepines by EMIT®, or with an accompanied history of benzodiazepine use, or both, were processed by the described procedure above for confirmation. To document the detection of α-hydroxytriazolam, a single 0.25 mg Halcion tablet was ingested and urine was collected eight hours later, the following morning.
Results and Discussion
Table 1 lists the ions that were monitored by SIM and the retention times of the individual compounds. The full spectrum of these compound derivatives contains only a few intense ions. This is a characteristic feature of MTBSTFA (5) and may be beneficial for the analysis of low concentrations of α-hydroxylalprazolam and α-hydroxytriazolam seen in urine after therapeutic doses. The ions in Table 1 have been monitored in our laboratory through many trial runs, always with blanks and controls, and these ions produce stable, consistent ratios without any interfering substances. Coefficient of variations (n=6) for the quantitation of a 200 ng/mL control were <5% and ±11% for within run and between run trials, respectively. The extraction efficiencies averaged 98% (range 91-107%) and the LOD and LOQ were both <10 ng/mL for all compounds. The high temperature GC conditions and short column length (15 m) are necessary to elute these compounds with symmetrical peak shape in a very short amount of time (within 6 min).

Quantitation and ion ratio data for the thirteen urine specimens were collected. Seven of these urines contain α-hydroxylalprazolam ranging in concentration from 49-1264 ng/mL (mean = 390). One specimen contained both α-hydroxyalprazolam (594 ng/ml) and oxazepam (476 ng/ml) oxazepam (3897 ng/ml) and temazepam (2685 ng/ml). Another one contained a very high concentration of 2-hydroxyethylfurazepam (2301 ng/ml). Five different specimens contained oxazepam ranging in concentration from 72-3897 ng/mL (mean = 1391). Temazepam (2-hydroxydiazepam or Restoril®) was present whenever oxazepam was in high concentration in all but one specimen. This specimen (oxazepam = 2620 ng/ml) had a self-reported history of clorazepate (Tranxene®). One specimen contained α-hydroxytriazolam (106 ng/ml) after the ingestion of a single 0.25 mg tablet of triazolam (Halcion®) eight hours earlier. Three specimens (49 and 62 ng/ml for α-hydroxyalprazolam and 106 ng/ml for α-hydroxytriazolam) were screened negative by the EMIT® assay but had absorbance readings that were elevated above the negative control. All other specimens were positive by EMIT®. All of the ion ratios used to confirm identity were within ±15% of those in Table 1. The usual limits of acceptability as positive are ±20%.

**TABLE 1**

| Retention time data and SIM Ions monitored (mean % relative abundance ± SD, n=10) of standards for the tert-butyldimethylsilyl derivatives of benzodiazepine compounds in urine. Base peak is the quantitation ion. |
|-----------------|-----------------|-----------------|-----------------|
| **Analyte**     | **Retention Time (Min)** | **Ions Monitored (m/z)** | **Relative Abundance (%)** |
| Oxazepam-D₅     | 3.00            | 462             | 464 (46 ± 1.0)   | 519 (28 ± 1.5) |
| Oxazepam        | 3.01            | 457             | 459 (46 ± 1.5)   | 514 (33 ± 2.3) |
| Temazepam       | 3.25            | 357             | 355 (43 ± 3.6)   | 283 (54 ± 3.7) |
| Lorazepam       | 3.50            | 491             | 493 (77 ± 1.7)   | 513 (69 ± 3.2) |
| 2-Hydroxyethyl- | 3.60            | 389             | 345 (11 ± 1.1)   | 391 (40 ± 0.9) |
| furazepam       |                 |                 |                 |                 |
| α-Hydroxy-       | 4.87            | 381             | 382 (30 ± 0.7)   | 383 (41 ± 2.1) |
| alprazolam      |                 |                 |                 |                 |
| α-Hydroxy-       | 5.33            | 415             | 416 (31 ± 1.3)   | 417 (72 ± 1.1) |
| triazolam       |                 |                 |                 |                 |

References
5. Regis Chromatography Catalog. p76.
ISOTOPE DILUTION MASS SPECTROMETRY DETERMINATION OF PLATINUM IN BIOLOGICAL FLUIDS OF A CANCER PATIENT

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Charlottesville, VA 22908

Introduction

Platinum (Pt) compounds are important anti-neoplastic drugs. Clinical evaluations of these drugs are dependent on the availability of precise, accurate and sensitive methods for Pt determination in urine, plasma ultrafiltrate and whole plasma. As an example, urine concentration of cisplatin is closely related to the non-protein bound concentration in blood. Plasma ultrafiltrate and whole blood concentrations of cisplatin provide information on free drug and protein bound drug, respectively. Since gas chromatography-mass spectrometry (GC-MS) is becoming more widely available in clinical laboratories, we have reported GC-MS methodology using lithium bis(trifluoroethyl)dithiocarbamate, Li(FDEDTC), as the chelating agent for the measurement of Pt (1). In the present work, Pt concentrations in plasma and urine from a cancer patient treated with cisplatin were determined by isotope dilution GC-MS and the results were compared to those obtained by the more commonly used method of electrothermal atomic absorption spectrometry (EAAS).

Experimental

The $^{192}$Pt-enriched Pt metal (50 atom % $^{192}$Pt, Oak Ridge National Laboratory) used as an internal standard for isotope dilution was dissolved in a minimum amount of aqua-regia at 70°C and the solution was taken to volume with dilute HCl. The internal standard solution was calibrated by reverse-isotope dilution GC-MS using natural Pt as the primary standard.

The known volume of the sample (1 mL of urine, 200 μL of ultrafiltrate or whole plasma) was mixed with a weighed amount of $^{19}$Pt internal standard solution followed by digestion with concentrated HNO$_3$ and concentrated H$_3$PO$_4$ (5:1, v/v) and 50% H$_2$O$_2$. Formic acid was used to destroy the residual HNO$_3$. The solution was evaporated to 500 μL and 500 μL of 10% HCl was added. Undigested lipids were removed by extracting with CH$_2$Cl$_2$ prior to metal chelate formation. The Pt(FDEDTC)$_2$ chelate was formed at pH 3 in an acetate buffer using a 20 mM solution of Li(FDEDTC). The chelate was then extracted into methylene chloride for GC-MS analysis.

Pt Isotope ratios were measured in duplicate, by injecting 1 μL of the chelate solution and monitoring the isotopic cluster corresponding to the molecular ion. The sample was injected, on column, into a 10 m DB-1 bonded-phase fused silica capillary column. Data were obtained in the selected ion monitoring mode using a Finnigan MAT-8230 GC-MS system operated in the electron ionization mode and quantitation was achieved by integrating the chromatographic peak areas.

In the EAAS method, Pt concentrations were determined using three different approaches. These were (i) calibration curve based on aqueous standards containing Pt in 10% HCl, (ii) standard addition and (iii) sample digestion as used for GC-MS followed by standard addition.

Results and Discussion

The overall precision of Pt isotope ratio measurements was found to be 1% when 10 ng of sample was injected. No measurable memory effect was observed when samples with isotope ratios differing by a factor of 80 were analyzed. A concentration value of 125 ± 6 (n=5) μg/L was obtained in the NIST urine reference material SRM-2670 compared to a recommended value of 120 ± 2 μg/L.

Table 1 shows the results obtained by isotope dilution GC-MS for the concentration of Pt in urine, ultrafiltrate and whole plasma samples from a cancer patient treated with cisplatin. For comparison, the
results of EAAS method are also included in the table. When compared to atomic absorption, good results were obtained for urine samples while there were significant differences in ultrafiltrate and plasma samples due to matrix effects in the atomic absorption assay.

Conclusions

Stable isotope dilution gas chromatography-mass spectrometry is a useful method for Pt determination in urine, ultrafiltrate and whole plasma. The present study was aimed at demonstrating the potential application of GC-MS method for clinical studies. A comparison of the results obtained by electrothermal atomic absorption spectrometry with GC-MS values emphasizes the need to critically evaluate EAAS and GC-MS methods for Pt determination in urine, ultrafiltrate and whole plasma samples.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample No.</th>
<th>Concentration of Pt (mg/L)</th>
</tr>
</thead>
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<tr>
<td></td>
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<td>GC-MS</td>
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<tr>
<td>Urine</td>
<td>1.</td>
<td>16.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>2.</td>
<td>2.0 ± 0.02</td>
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<tr>
<td></td>
<td>3.</td>
<td>1.9 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>4.</td>
<td>1.5 ± 0.02</td>
</tr>
<tr>
<td>Whole</td>
<td>1.</td>
<td>6.8 ± 0.2</td>
</tr>
<tr>
<td>Plasma</td>
<td>2.</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>3.</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>Ultra</td>
<td>1.</td>
<td>0.8 ± 0.03</td>
</tr>
<tr>
<td>Filtrate</td>
<td>2.</td>
<td>0.4 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>3.</td>
<td>0.2 ± 0.01</td>
</tr>
</tbody>
</table>

Note: EAAS (I) refers to the use of calibration curve based on aqueous solutions; EAAS (II) refers to wet digestion and standard addition.

Reference

Garlic-borne volatile compounds were monitored by breath analysis of human subjects in a time course study following ingestion of freshly minced raw garlic. Subjects ingested garlic at a dosage which varied between 40-200 mg per Kg of body weight and at various time intervals thereafter exhaled one liter of breath through an adsorbent trap containing Tenax-TA and Carbotrap. The adsorbent traps were then analysed using Short Path Thermal Desorption GC-MS. Quantification was achieved by GC with flame ionization detection using 2,5-dimethylthiophene as an internal standard. Linear calibration curves ($R^2 = 1.0$) were obtained by plotting the ratio of each specific analyte peak area / peak area of 2,5-dimethylthiophene versus concentration of analyte with a dynamic range covering four orders of magnitude. A total of six garlic-borne volatile compounds were found consistently in all subjects ingesting raw garlic. Four of these compounds (allylmethylsulfide, diallylsulfide, allylthiol and diallyldisulfide) are well known character species of garlic aroma. Surprisingly, the other two compounds (p-cymene and d-limonene) are terpenoid species which have not as of yet been reported as occurring in garlic. Figure 1 is a mass chromatogram for ions characteristic of garlic-borne phytochemicals found in the breath 1.5 hours following ingestion. The breath concentration clearance curves for d-limonene, p-cymene and several allylic sulfur compounds are shown in figures 2, 3 & 4 respectively. The individual appearance and elimination curves for these phytochemicals were found to differ significantly suggesting the experimental observations are perhaps related to the pharmacokinetic behavior of the individual compounds. All elimination curves were biphasic in appearance. Levels of methylallylsulfide, allylthiol, diallylsulfide and diallyldisulfide were initially high but were found to drop precipitously within in a short period of time (1-3 hrs). The slope of the elimination curve then changed dramatically, exhibiting a plateau which was maintained throughout the sampling interval (1-24 hours). We believe that the initial high levels and rapid decline of the allylic sulfur compounds breath concentrations are related to their elimination from the oral and pharyngeal regions where they are present as residues immediately after ingestion. However, the plateau portion of the clearance curve most likely results from bloodstream elimination of these compounds via gas exchange in the lungs. The slope change of the clearance curves certainly coincides with the time expected for digestion and bloodstream absorption to occur. The breath concentration curves for the terpene species were very different than those observed for the allylic sulfur compounds. The breath concentrations of terpenes were initially low or nonexistent but between 1-4 hours after ingestion, the levels of the two terpenes rose dramatically, perhaps reflecting their absorption from the intestinal system into the bloodstream as the garlic is digested. With additional research this methodology may eventually prove useful as a non-invasive analytical technique for measuring bloodstream concentrations of volatile or semivolatile food-borne chemicals. This principle has already been successfully exploited in the breathalyzer test for alcohol. We acknowledge the Center for Advanced Food Technology (CAFT) Mass Spectrometry facility for providing analytical support. CAFT is an initiative of the New Jersey Commission on Science and Technology. This is NJAES publication number S-10569-2-91.
FIG. 1. MASS CHROMATOGRAMS FOR IONS CHARACTERISTIC OF GARLIC-BORNE PHYTOCHEMICALS ON THE BREATH AT T=1.5 HOURS AFTER INGESTION OF FRESHLY MINCED RAW GARLIC

FIG. 2. BREATH CONCENTRATION OF D-LIMONENE VS TIME AFTER INGESTION OF FRESHLY MINCED RAW GARLIC

FIG. 3. BREATH CONCENTRATION OF p-CYMENE VS TIME AFTER INGESTION OF FRESHLY MINCED RAW GARLIC

FIG. 4. BREATH CONCENTRATIONS OF VARIOUS ALLYLIC SULFUR CONTAINING GARLIC-BORNE PHYTOCHEMICALS VS TIME AFTER INGESTION OF FRESHLY MINCED RAW GARLIC

THE CONCENTRATION OF METHYLALLYL SULFIDE VS TIME FOR FRESH GARLIC (SUBJECT 1)

THE CONCENTRATION OF DIALLYL SULFIDE VS TIME FOR FRESH GARLIC (SUBJECT 1)

THE CONCENTRATION OF ALLYLTHIOL VS TIME FOR FRESH GARLIC (SUBJECT 1)
Metabolites of U-86170F Present in Rat Urine Following Oral Administration as Determined by LC-MS and GC-MS

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U-86170F (5-(dipropylamino)-5,6-dihydro-4H-imidazo[4,5,1-γ]quinolin-2(1H)-one) is a compound under investigation as a potential neuroleptic. The responses observed in animal model screens following oral administration indicate that oral bioavailability is poor. One possible explanation is rapid metabolism of U-86,170 by the liver following oral administration. To further investigate this observation we have employed thermospray (TSP) mass spectrometry (MS) and high resolution gas chromatography (HRGC) MS to characterize metabolites of U-86,170 in rat urine, with the objective of understanding both the extent of metabolism of U-86,170 and the major pathway(s) important in any observed metabolism. 'Metabolic profile' information is often of value in designing and selecting alternate lead-finding compounds which retain activity and exhibit increased bioavailability.

Metabolites of U-86,170 present in the urine of rats following oral administration as determined by TSP-LC-MS and HRGC-EI-MS following chemical derivatization are reported.

Sample Extraction and Purification: Urine samples were extracted using ODS (Sep-Pak®) cartridges. No further purification was attempted. Treatment: 20 mg/kg U-86,170F p.o. Samples: Pre-dose (control) and 0-4 hour urine samples. LC-MS: Perkin-Elmer (Norwalk, CT), Vestec (Houston, TX) and Nermag (R1010, Houston, TX). Chromatography: 0.1 M ammonium acetate, Ph = 4.0 (solvent A) and acetonitrile (solvent B). Linear gradient from 100% A to 70:30 A:B or 50:50 A:B in 20 minutes. Perkin Elmer 3 mm x 8 cm Percosphere C-18 column. TSP: Block 258 ºC, vaporizer 234 ºC, repeller 120 volts, discharge off. GC-MS: Hewlett Packard (5890) and Nermag (R1010) equipment. Chromatography: J&W Scientific 15 m DB-5, 0.25 mm I.D., 0.25 μm film thickness, Helium, splitless injection, injector 250 ºC, oven 100 ºC, increased to 310 ºC at 25 ºC/min.

The positive ion TSP mass spectrum of U-86,170, U-77,567 (5-propylamino analogue) and U-77,292 (5-amino analogue) consisted only of the pseudo-molecular ions (MH+, m/z 274, 232, 190), a common observation using positive ion TSP ionization. U-77,567 and U-77,292 were of interest since they were hypothesized to be metabolites of U-86170. These data are summarized in Figure 2. Several differences were noted in the TSP analysis of pre-dose (control) and 0-4 hour (drug) samples. TSP analysis indicated that some parent drug is excreted unchanged in the urine (0-4 hour collection period) and that the major metabolite present at this time is the N-dealkyl metabolite (U-77,567). Metabolites 16 daltons greater in molecular weight than the parent drug were observed as a metabolite 16 daltons greater than the molecular weight of the N-dealkyl metabolite. The primary amine (U-77,292) was not observed using TSP analysis. Glucuronide and sulfate conjugates of the oxidized metabolites were either not present or below the level of detection.

The TSP data indicated that N-dealkylation is apparently the major, or one of the major metabolic pathways involved in U-86,170 biotransformation in the rat. Also, hydroxylation is apparently an important metabolic route. It was decided that chemical derivatization and high resolution gas chromatography-electron ionization-mass spectrometry (HRGC-EI-MS) would be employed to determine the general (i.e., aryl vs. aliphatic) sites of oxidation. Samples were dried and reacted with BSTFA at 80 ºC for 30 minutes. The electron ionization (EI) mass spectrum was obtained for the TMS derivative.
of U-86,170. To further characterize the hydroxylated metabolites of U-86,170 using EI-MS it was necessary to demonstrate the origin of one or two key fragments in the EI mass spectrum of the TMS derivative that were characteristic of an intact and unchanged dipropylamino moiety. EI mass spectra of the TMS derivative of U-86,170 would be expected to contain a characteristic ion at m/z 126 (C_{6}H_{7}N^+-(CH_{3}-CH_{2}-CH_{3})_{2}) and this ion was observed. Also, shifts of 14 daltons in the EI mass spectrum of the propylbutylamino and dibutylamino structural analogues of U-86,170 were observed. High resolution MS analysis of the TMS derivative of U-86,170 confirmed this hypothesis. Thus, the m/z 126 ion is indicative of an intact 5-dipropylamino moiety. Three distinct peaks were observed for the drug related sample in the reconstructed mass chromatogram for m/z 433 (M* of the di-TMS derivative of a hydroxylated metabolite of U-86,170) that were not present in the control sample. The three metabolites were observed to have intense fragment ions at m/z 126, suggesting that the dipropylamino groups are unchanged in these metabolites. Aryl hydroxy structures are plausible, although the presence of metabolites are not ruled out by these data. The requirement for GC-MS analysis demonstrates the structural elucidation limitations of TSP-MS analysis.

Figure 2. TIC and selected reconstructed mass chromatograms for pre-dose and control rat urine samples as determined by thermospray analysis.
INTRODUCTION

MDL 72,222 (8-methyl-8-azabicyclo[3.2.1]octan-3-yl 3,5-dichlobenzoate) is a potent and highly selective serotonin (5-HT₃) M-receptor antagonist which is currently under evaluation as an antiemetic drug to be used as an adjunct to cancer chemotherapy. Some of the metabolites show activity and account for a large portion of the C₁₄ dose. This GC/MS method is an improvement over a previous one using a less specific detector (electron capture). We report here a specific and sensitive method for the quantification of parent drug and four metabolites (Fig. 2); N-desmethyl-MDL 72,222 (1), 3,5-dichlobenzoic acid (2), glycine conjugate of 2 (3), and MDL 72,222-N-oxide (4) in citrated monkey plasma using negative chemical ionization GC/MS.

A multistep procedure was necessary to extract and derivatize all analytes due to their wide variation of polarity. Quantification of analytes in samples was made by interpolation of standard curves. The standard curves were split into two linear segments of either 0.5 to 10 and 10-75 ng/mL (metabolites 1 and 3) or 1-20 and 20-150 ng/mL (MDL 72,222 and metabolites 2 and 4).
RESULTS AND DISCUSSION

Validation was done in monkey and dog plasma. Extraction efficiencies ranged from about 60-90%. Intraday precision values were less than 10% RSD and accuracy values were in the range of 92.0-110%. Similarly, the Interday precision values were less than 14% RSD and accuracy values were in the range of 87.6-116%.

Pharmacokinetic studies to determine the plasma concentrations of MDL 72,222 and metabolites in dogs and monkeys given a single iv dose and in dogs given multiple iv doses of MDL 72,222 were performed. Plasma concentration-time profiles from the single dose study were used to determine various pharmacokinetic parameters and showed a nearly quantitative accounting of $^{14}$C. The multiple dose study was used to assess the following; dose-proportionality, accumulation, and steady-state conditions. A plot of drug and metabolite concentrations in monkey plasma is shown below. The animal given a single 5 mg/kg intravenous dose of MDL 72,222.

PLASMA CONCENTRATION-TIME PROFILES OF MDL 72,222 (○), 1 (△), 2 (○), 3 (○), 4 (△) IN A MONKEY GIVEN A 5 MG/KG INTRAVENOUS DOSE OF MDL 72,222.
MASS SPECTROMETRIC CHARACTERIZATION OF THE METABOLITES OF
DEOXYSPERGUALIN (NKT-01) IN HUMAN URINE

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Y. Hashimoto, T. Saino, T. Nakamura* and T. Takeuchi**

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Introduction

Deoxyspergualin (NKT-01 : (+)-1-amino-19-guanidine-11-hydroxy-4,9,12-triazanona-
decane-10,13-dione 3HCl) (Table 1) is a synthetic spermidine derivative with strong
immunosuppressive and antitumor activity [1,2]. The present compound is currently
under evaluation for potential use as a therapeutic agent against acute organ
rejection after transplantation [3,4]. In preclinical studies, we have demonstrated
that urinary excretion plays an important role for the elimination of NKT-01 in rat
and dog. The present communication reports an isolation and mass spectrometric
characterization of the urinary metabolites of NKT-01 in human following intravenous
administration.

Experimental

Apparatus

Fast atom bombardment-mass spectrometry (FAB-MS) was performed using a
MM-ZAB HF (VG Analytical). Samples were dissolved in a glycerol or thiglycerol
as a matrix and ionized by bombardment with a xenon atom beam. Gas chroma-
tography (GC)/MS analyses (electron ionization) were run on a Hitachi M-80B
GC/MS equipped with a fused silica capillary column using helium as carrier gas [5].
High performance-liquid chromatographic (HPLC) system consisted of a Shimadzu
LC-6A pump, SPD-6A ultra violet detector and RF-535 fluorometric detector, and
Reodyne 7125 injector. The column was a reversed phase Cosmosil 5C18-P (15cm x
4.6mm i.d.). The mobile phase consisted of 0.01M phosphate buffer (pH 3)-aceto-
nitrile (91:9) containing 5mM sodium 1-pentanesulfonate as a modifier [6].

Sample preparation

Samples of urine from patients were collected into 0.5M NaH2PO4 for up to
24 hours after NKT-01 administration. Metabolites in urine were extracted and
purified by CM-Sephadex C-25 column chromatography (Na+ form) and subsequent
HPLC. Purified metabolites were converted to the methyl ester (ME)-o-methyl
derivatives (FAB-MS) and the pyrimidine derivatives (GC/MS) [5].

Results and Discussion

Extraction and fractionation of the metabolites by CM-Sephadex C-25 column
chromatography and subsequent purification of the eluate by reversed phase-HPLC
resulted in the isolation of six metabolites in addition to intact NKT-01 from human
urine. Characterization of the isolated metabolites was accomplished by comparing
the retention time in HPLC and their FAB mass spectra with authentic reference
compounds. Mass spectral data of the identified metabolites are shown in Table 1.
FAB mass spectra of the metabolites were characterized by the appearance of
protonated molecular ions ([MH]+) after methylolation with hydrogen chloride in
methanol as shown in Fig.1. Derivatization of each metabolite with acetylacetone
resulted in the formation of the pyrimidine derivative of GUS-4 (m/z 250, [M]+,
GC/MS), suggesting that all metabolites identified possessed 7-guaninedeheptanamide
moiety in the molecule. Of these metabolites, GUS-10 and GUS-14 were compounds
with carboxyl functional group. The metabolite possessing aldehyde functional
group (GUS-8) was also identified as one of the major urinary component.

In vitro biotransformation of NKT-01 by calf serum gave GUS-8 as the sole
component. The formation of GUS-8 may be catalyzed by amine oxidase. These
results suggested that amine oxidase may be a promising key enzyme which is
responsible for oxidative degradation of spermidine moiety in NKT-01 in vivo.
Similarity in the metabolic process between NKT-01 and spermidine was also
strongly indicated from the structures of metabolites.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

References


Table 1  Mass spectral data and structures of NKT-01 and its metabolites

<table>
<thead>
<tr>
<th>Structures</th>
<th>FAB-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>m/z 402 ([MH]+)</td>
</tr>
<tr>
<td></td>
<td>(o-methyl)</td>
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<tr>
<td>GUS-8</td>
<td>m/z 462 (GC/MS)</td>
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<td>(butoxime-PFB)</td>
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<td></td>
<td>([M–butanol]+)</td>
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<td>GUS-10</td>
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<td>(ME-o-methyl)</td>
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<td>m/z 279 (GC/MS)</td>
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<tr>
<td></td>
<td>(M+ : Et–pyrimidine)</td>
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Fig.1 Mass spectrum of the ME-o-methyl derivative of GUS-14
Quantitative GC/MS Determination of Arachidonic Acid
Released from Cultured Human U937 Cells

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P.O.Box 1005, Pointe-Claire-Dorval, Quebec, Canada, H9R 4P8

Oxygenated arachidonic acid metabolites are mediators of broncho-constriction, vaso-constriction and inflammatory reactions, and are implicated in the pathology of diseases in man, e.g., asthma, arthritis, psoriasis and inflammatory bowel disease. The rate limiting step in the biosynthesis of such mediators as leukotrienes, prostaglandins and PAF is believed to be the hydrolysis of arachidonic acid from the sn-2 position of membrane phospholipids within the cell by one or more phospholipase A2 (PLA2) enzymes. Because of the direct involvement of neutrophile and other leucocytes in the inflammatory processes, there is an increasing interest in the pharmacological manipulation and characterization of arachidonic acid releasing PLA2 activity in these cells. Many studies have reported the metabolism of 3H-arachidonic acid in stimulated cells, including the hydrolysis of arachidonyl labelled phospholipids. However, there are few reports of direct measurements of arachidonic acid release from endogenous cell phospholipids. Our objective is to study arachidonic acid release in differentiated human U937 cells as a model system to investigate the role of PLA2 and its physiological and pharmacological regulation in inflammatory cells.

Existing methods for arachidonic acid quantification have relied mainly on gas chromatography with either flame ionization or electron capture detector. In this study, the methodology for quantitative determination of arachidonic acid release in differentiated human U937 cells is based on the formation of pentafluorobenzyl ester of arachidonic acid, and analysis by GC/MS. A known amount of 3H arachidonic acid was used as internal standard and spiked into each sample, followed by liquid-liquid extraction with Dole's method (heptane, isopropanol, H2SO4) to extract free fatty acids. The free fatty acids were then derivatized to their pentafluorobenzyl esters, and resuspended in 1 mL of dodecane (or tetradecane). Resonance electron capture ionization (CH4) was employed with selected ion monitoring of the predominant carboxylate anions, resulting from the loss of pentafluorobenzyl radical. The detection limit of arachidonic acid was 20 pg/mL.

The arachidonic acid level was very low in resting cells (~0.8 ng/10^6 cells), and was found to increase rapidly upon Ca^2+ ionophore A23187 stimulation (reached maximum at 3 minutes stimulation, ~120 ng/10^6 cells), and decrease thereafter. DMSO differentiated U937 cells gave a greater arachidonic acid response (~170 ng/10^6 cells) upon A23187 stimulation compared with non-differentiated U937 cells (~42 ng/10^6 cells). Furthermore, The amount of each of 10 fatty acids in differentiated human U937 cells was measured, and we found that arachidonic acid increased upon A23187 stimulation more than any other fatty acid, to become the major free fatty acid present. We believe that this method provides a useful and specific assay for the study of arachidonic acid release and PLA2 activity in whole cells.

References:
   (c) H. Heckers, F.W. Melcher and V. Schloeder J. Chromatogr. 136 (1977) 311.
Table 1. Free Fatty Acids in Human U937 Cells

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Resting cells (ng/8x10^6 cells)</th>
<th>3 min. A23187 Stimulated cells (ng/8x10^6 cells)</th>
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<td>12:0</td>
<td>1.9 ± 0.97</td>
<td>3.0 ± 0.32</td>
</tr>
<tr>
<td>14:0</td>
<td>4.2 ± 0.80</td>
<td>15.5 ± 0.88</td>
</tr>
<tr>
<td>16:1</td>
<td>0.4 ± 0.16</td>
<td>12.0 ± 0.51</td>
</tr>
<tr>
<td>16:0</td>
<td>&lt; 1</td>
<td>96.8 ± 7.34</td>
</tr>
<tr>
<td>18:3</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>18:2</td>
<td>1.3 ± 0.17</td>
<td>12.4 ± 0.95</td>
</tr>
<tr>
<td>18:1</td>
<td>5.8 ± 0.46</td>
<td>98.8 ± 11.98</td>
</tr>
<tr>
<td>18:0</td>
<td>&lt; 1</td>
<td>181.3 ± 6.64</td>
</tr>
<tr>
<td>20:4</td>
<td>0.5 ± 0.08</td>
<td>430.2 ± 18.77</td>
</tr>
<tr>
<td>22:6</td>
<td>0.5 ± 0.03</td>
<td>33.6 ± 2.24</td>
</tr>
</tbody>
</table>

Free fatty acids were measured in 5 replicated, each containing 8x10^6 cells. Quantification of each fatty acid was carried out by resonance electron capture ionization and selected ion monitoring of the 11 carboxylate anions: CH₃(CH₂)₇-COO⁻, including ²H₄-20:4 as internal standard.
PLASMA SERINE IN SCHIZOPHRENICS AND CONTROLS MEASURED BY GC-MS

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In a series of studies on more than 350 psychotic (including schizophrenics) and non-psychotic subjects, higher plasma serine concentrations (PSC) in the psychotics have consistently been observed [2]. Diminished serine hydroxymethyltransferase (SHMT) activity in the plasma of psychotic subjects has also been found [3]. Since SHMT provides the major degradative pathway for serine metabolism, the high PSC of psychotic and schizophrenic subjects is likely to be due to decreased activity of the SHMT enzyme in the liver. Neuroleptic drug usage, which is most often a differentiating variable between psychotics and non-psychotics was minimized in this study. That is, psychotics were placed in a research ward, consuming a balanced diet and off neuroleptics for three or more weeks prior to blood withdrawal. In any case, neuroleptics tend to reduce PSC in psychotic subjects.

It has been suggested that the high PSC observed in previous studies for psychotics is due to technical problems in the gas chromatographic (GC) assays where some other substance is co-eluting with serine [4]. By using GC/MS, whereby accurate identification and quantification of substances can be achieved, the likelihood of a co-eluting compound can be ruled out (see Figure 1 for TIC chromatograms of amino acid (AA) standards and plasma AA samples; Figure 2 shows the corresponding mass spectra for serine, glycine, and norleucine).

In this study a VG Trio-1 GC/mass spectrometer (MS) was used. N(O)-heptafluorobutyryl isobutyl ester derivatives of the plasma and standard amino acids were prepared for GC/MS analysis. Norleucine was used as an internal standard for quantitation (see Figure 3 for the glycine and serine calibration curves). In general, the plasma serine concentration (PSC) of schizophrenics was found to be 104.0 nmoles/mL (± 19.4) and was determined to be 75.3 nmoles/mL (± 13.8) for controls (see Figure 4 for PSC scatter-plot). Since glycine is the by-product of serine metabolism through SHMT activity, one would expect the plasma glycine concentrations (PGC) to be lower for schizophrenics vis-a-vis controls. It is interesting to note that the PGC were also found to be higher for schizophrenics as compared to controls. The PGC for schizophrenics was found to be 214.4 nmoles/mL (± 49.7) and determined to be 164.1 nmoles/mL (± 43.3) for controls (see Figure 5 for PGC scatter-plot).
In two previous studies, we had found no significant difference between the PGC of psychotics and non-psychotics. Whether this discrepancy is due to the fact that the present group of schizophrenics was off medications is at this time unknown. We also used heptfluoroacbutyric anhydride to derivatize the amino acids instead of trifluoroacetic anhydride as reported in our previous studies. Macciardi, et al. have also observed high PGC as well as high PSC in their schizophrenic subjects [5]. In a study of the autopsied temporal lobes, significantly higher serine and glycine concentrations in the brain samples of schizophrenics compared to controls was reported [6]. The major degradative pathway of glycine is via the glycine cleavage complex (GCC) [7], which in the mitochondria is closely coupled to SHMT [8]. Glycine may also be decarboxylated by SHMT in the presence of lipoic acid [9]. Whether there is a concomitant deficiency in the GCC, which would raise glycine concentrations in schizophrenics or the increased glycine levels are also due to the deficient activity of SHMT, is at this time unknown. That the metabolism of these two AA is closely linked is suggested by a significantly high correlation (r = 0.675, p < 0.0002) between PSC and PGC of both controls and schizophrenics.

DETERMINATION OF MEVALONIC ACID IN SERUM BY MASS SPECTROMETRY FOR THE STUDY OF CHOLESTEROL SYNTHESIS INHIBITION

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Recent studies have shown a correlation between elevated serum cholesterol levels and risk of cardiovascular disease. Prior to the development of therapeutic agents, the biological pathway of cholesterol synthesis was studied and key steps in the synthesis identified. Subsequently several compounds were developed to inhibit these key steps. Both pravastatin and lovastatin, for example, inhibit the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonic acid.

There is also an interest in inhibiting other steps in the synthesis pathway. To monitor the effect of a compound that would inhibit the conversion of mevalonic acid to 5-phospho-mevalonic acid, it would be useful to have a method capable of detecting increases in mevalonic acid levels. This has previously been accomplished by an enzymatic method and several gas chromatography (GC)-mass spectrometry (MS) methods. The enzymatic assay requires the use of $^{32}$P labeled compound requiring stringent safety precautions. The GC-MS methods, while selective and sensitive, are all encumbered by extensive extraction procedures.

The work reported here describes a simple and expedient method for the quantitative determination of mevalonic acid in human and rat serum using a much simpler extraction procedure together with either GC-electron impact (EI) MS or GC-Positive Ion Chemical Ionization (PICI) MS with ammonia as the reagent gas. The extraction involves a simple protein precipitation with ethyl acetate which isolates the compound in the acid form. The compound was then trimethylsilated (TMS) and injected onto a Restek Rtx-5 capillary column contained in a Hewlett-Packard 5990 GC and analyzed by electron impact or chemical ionization mass spectrometry. The EI experiments were performed on a Hewlett-Packard 5970 MSD, the CI experiments on a VG Trio-2. Full scan spectra obtained with each of the two ionization methods are shown in Figures 1 and 2. For purposes of quantification, the m/z 247 and 250 (tri-deuterated internal standard) ions were monitored in EI; the m/z 275 and 278 ions in CI.

As shown in Figures 3 and 4, both the EI and CI methods provide linear standard curves over the concentration range of 10 to 200 ng/ml. Accuracy and precision of the methods were assessed by running QC samples in quadruplicate at three concentrations on three different days. At each level, predicted concentrations deviated from nominal by less than 15% for the EI method and by less than 5% for the CI method. The methods have been used successfully to evaluate the effect of cholesterol lowering agents in several different studies.

Structure of trimethylated mevalonic acid

\[
\text{CH}_3\text{OTMS} \quad \text{OTMS} \quad \text{OTMS}
\]

Structure of trimethylated mevalonic acid
The following represent full scan spectra of trimethylsilated mevalonic acid

Figure 1. El Spectrum

Figure 2. Cl Spectrum

Calibration curves for both methods are shown in the figures below.

Figure 3. El Standard Curve

Figure 4. Cl Standard Curve

\[ y = 0.010483697x + 0.07497522 \]

\[ y = 0.013574129x + 0.097115817 \]
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MASS SPECTROMETRY IN FERMENTATION BATCH PROCESSING OF ANTIBIOTICS

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Ardacin (AAD216 Complex) is a fermentation product that belongs to vancomycin family of antibiotics. They are produced by a new genus of Actinomycetales, Kibdelosporangium aridium (ATCC 39323). The complex is currently being developed as a growth promotant for food producing animals.

The complex consists of ten known glycopeptides with each of them having the same heptapeptide skeleton that constitute approximately 78% of the activity. The product impurities constitute the remaining 22%. The fermentation and the purification of the broth takes place in several different batches at the manufacturing plant. Therefore, the nature and composition of impurities may vary depending on the production batch. In this presentation, the application of mass spectrometry to characterization of impurities in different batches into structural classes will be discussed. The characterization involved; separation of the glycopeptide complex from impurities, FAB/MS screening, chemical degradation and derivatization followed by further analysis using FAB, EI, and GC/MS. The characterization of any given batch includes component separation based on molecular size, screening by fast atom bombardment mass spectrometry, chemical degradation and derivatization of components other than that constitute 78% activity of the complex and further characterization by mass spectrometry. Several structural classes, such as a peptide with unique identity, glycolipids and carbohydrates have been identified and their spectral data will be presented.

Experimental

Separation of the glycopeptide complex from impurities was carried out using a Bio-Gel (P-4) column (80cm x 2.6cm). The crude raw material was applied to the column in a solution of ammonium bicarbonate and washed with a solution of the same. Approximately 100-102 x 15 ml fractions were collected for each run and lyophilized to dryness prior to analysis. The chemical degradation involved; hydrolysis (6N HCl, 18 hrs), and methanolysis. Derivatization such as methylation, acetylation after NABH₄ reduction was carried out. All mass spectra were obtained with a VG 70/250SE mass spectrometer. A VG 11-250J data system was used to acquire and process all data. For FAB/MS, a standard FAB ion source and an Ion Tech fast atom gun was used. The accelerating voltage was maintained at 8 kV while 8 keV xenon atoms at a discharge current of 1 mA were used to bombard the sample. Monothioglycerol/oxalic acid mixture, m-nitrobenzyl alcohol and glycerol were used as matrices. For GC/MS, a fused silica column (CP SIL 5CB, 25 cm x 0.25 mm) was used. The column oven was programmed; 90°C for 2 min., ramped at 10°C/min. to 270°C and held at the final temperature for 5 min.

Results and Discussion

The initial screening of the bio-gel fractions was helpful in disseminating the impurities from the glycopeptide complex. A typical FAB/MS spectrum of the glycopeptide complex (figure 1) is given in figure 2. Extensive characterization of this complex has been reported elsewhere¹. The molecular ion cluster consists of the average molecular ions (M+H)+ of m/z 1787, 1801 and 1815 derived from the major components of the complex. The FAB/MS screening also indicated molecular ions with m/z 16 daltons higher than the corresponding major components probably due to
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hydroxylated ardacins. A molecular ion of m/z 1051 was detected in several early eluting Bio-Gel fractions (figure 3). After accurate mass determination, elemental analysis and degradation chemistry, a probable structure of a hexapeptide having the ardacin aglycone skeleton without the amino acid marked G was assigned. The main portion of the other Bio-Gel fractions consisted of glycolipids containing long chain hydrocarbons, carbohydrates and sialic acids.

In conclusion, mass spectrometry can be successfully applied to characterize fermentation products and their impurities; thereby, variations of constituents, if any, in different batches can be determined prior to utilization of the final product in developmental studies.

Acknowledgement

The authors wish to acknowledge the assistance given by David Hallman in this poster presentation.

THE MASS SPECTROMETRY CUTOFF FOR THE COCAINE METABOLITE, BENZOYLECGONINE

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Introduction
The final criteria for U.S. Federal Workplace Drug Testing Programs was set forth in 1988 (1). Under these guidelines the benzoylecgonine cutoff concentrations for screening (300 μg/L) and confirmation (150 μg/L) were established based primarily on the experience of the Department of Defense and the testing of military personnel. The state of the art in drug detection and confirmation was also considered. Provision was made for change in these guidelines as technology advanced and methods improved.

A reduction in the Benzoylecgonine cutoff levels requires both an improvement in screening and confirmation ability, the need for such a reduction to better produce a drug free workplace and assurance that this will not produce false positives. As previously published, commercially available screening methods using EMIT and Fluorescence Polarization Immunoassay currently exist which permit limits of detection at a 95% confidence level of 24 μg/L and 30μg/L, respectively (2,3). During 1990 at Toxicology Testing Service Inc., EMIT screening of employment samples at a cutoff level of 50 μg/L and full scan GC/MS confirmation produced 41% confirmed positives above the federally mandated cutoff level of 150 μg/L, 44% between 20 and 150 μg/L, 8% below 20 μg/L and 7% negatives. More than half the samples containing cocaine metabolite were reported as negative with the current confirmation test level. A recent exaggerated test during which subjects were required not to wash their hands during a whole day to determine the upper benzoylecgonine urine levels resulting from exposure to contaminated money, found a maximum screening level of 72 μg/L (4). As pointed out in Sunshine's book (5) a better method of confirmation is still needed if a lower cutoff is to be implemented.

Experimental
Standards were prepared in negative urine at 2, 5, 8, 20, 150 and 300 μg/L levels. Three mL samples were analyzed after addition of 60 ng of D-3 benzoylecgonine in dimethylformamide as an internal standard. Samples were extracted after adding 1 mL of 1.5M Potassium Phosphate Dibasic with 4 mL of 20% isopropanol in methylene chloride by rocking for 15 minutes. Samples were centrifuged, if necessary, and the bottom, organic layer was removed with a Pasteur pipet and evaporated to dryness at 65°C under a stream of nitrogen. Derivatization to prepare propyl benzoylecgonine was done by heating the evaporated sample at 65°C for 10 minutes after addition of 200μL, 2% tetramethylammonium hydroxide in acetonitrile and 40μL iodopropane. After cooling to room temperature 1 mL, 0.2N HCl was added and the sample extracted by vortexing 15 seconds with ethyl acetate. The top, ethyl acetate layer was discarded, 1 mL, 1.5M potassium phosphate dibasic added and the sample extracted with 1.2 mL of 50/50 hexane in methylene chloride by vortexing 30 seconds. The top, organic layer was then removed and evaporated to dryness at 65°C under a stream of nitrogen, reconstituted with 10 to 20 μL of isooctane and analyzed. One or two μL samples were injected using a LEAP Technologies autoinjector into a Grob Splitless port and a 12 meter DB-5 capillary column with a temperature program of 150-300 at 18 °C per minute. A Finnigan 4000 (with PPINICI) GC/MS was used to obtain mass spectra. Quantitation was accomplished using a linear calibration curve prepared from the 210/213 (Benzoylecgonine/methyl-D3 Benzoylecgonine) integrated ion ratios obtained from the full scan (52-400) mass spectral data acquired.
Results and Discussion

Analysis of the linear calibration curve (Figure 1) obtained from the standards in triplicate ($r^2 = 0.998$) using the method described by de Kanel et al. (3) and Hubaux and Vos (6) yielded a limit of detection of 9.4 μg/L at the 95% confidence limit. A limit of quantitation of 32 μg/L was determined assuming an acceptable error of ±20% at the 99% confidence level. Chromatograms were clean with the benzoylecgonine derivative producing the major peak down to a level of 5 μg/L (Figure 2). The mass spectra obtained at the 5 μg/L and 150 μg/L levels were in excellent agreement (Figures 3 and 4). Substantially lower benzoylecgonine cutoff levels are now justified.


The author wishes to thank Toxicology Testing Service, Inc. for partial support of this work.
A Method for the Detection of Urinary Synthetic Corticosteroids by Gas Chromatography-Mass Spectrometry

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The use of synthetic corticosteroids by athletes has been banned except for treatment as short-term anti-inflammatories or analgesics involving topical preparations, inhalation therapy or local injections. This has created the need for a routine screening and confirmation procedure capable of detecting a wide variety of synthetic corticosteroids.

Various analytical techniques generally involving combinations of high performance liquid chromatography, gas chromatography and mass spectrometry have been developed. However, most of the methods described in the literature detect only a few corticosteroids per analysis and would be difficult to perform routinely on a large scale. A simplified, routine and automated procedure for the detection of synthetic corticosteroids and their metabolites can be performed by modifying current analytical procedures using an automatic sample injection system with a gas chromatograph coupled to a mass spectrometer. Screening results are printed utilizing a computerized data reduction system.

An aliquot of urine with internal standard was passed through a C18 Sep-Pak (Waters) cartridge followed by water and methanol to extract the corticosteroids from the urine matrix. The dried methanolic extract was subjected to enzymatic hydrolysis using 0.2 mol/L acetate buffer (pH 5.2) and a crude solution of Helix pomatia for two hours in a 60°C waterbath. The corticosteroids were then extracted into diethyl ether with the addition of potassium carbonate. To protect the integrity of the thermally labile C17 side chain, the vulnerable keto and hydroxyl groups were converted to their methoxime and trimethylsilyl ether derivatives respectively using a modified technique first developed by Horning, et al.

Derivatization involved a two step process. Following derivatization with MOX (methoxyamine HCl) in pyridine for 3 hours at 90°C and removal of the pyridine, the extract was reacted with TSIM (N-trimethylsilylimidazole) overnight for 18 hours at 105°C. These extreme conditions were necessary to force complete derivatization of any hindered hydroxyl groups especially those encountered in compounds with C16 methyl group substitutions such as dexamethasone illustrated in figure 1.

Figure 1 Dexamethasone MOX-TMS derivative
The derivatives were injected using splitless mode by a Hewlett-Packard (HP) 7673A Automatic Liquid Sampler onto a 20 m DB-1 (J & W Scientific) polymethyl siloxane capillary column fitted into a HP 5890 Gas Chromatograph coupled to a HP 5970 Mass Selective Detector (MSD). The MSD was tuned according to the manufacturer's specifications and was operated in the selected ion monitoring (SIM) mode using 50 ms dwell times. Data reduction and interpretation were simplified by a macro or program which produced a printed report displaying the SIM data for each corticosteroid and/or its metabolite. A presumptive positive was indicated by matching peaks for each of the selected ions at the proper retention time.

Detection limits were determined at approximately 10 - 25 ng/mL. Recovery studies using this method indicated an average of 70 - 95% for most of the synthetic corticosteroids analyzed except for triamcinolone which had a recovery of approximately 50%.

Excretion studies using urine samples from patients on corticosteroid therapy were performed to determine if the parent compound and in particular any metabolites could be detected. Prednisolone, the active form of prednisone, was detected in patients on prednisone therapy. Both the parent compound and their major 6ß-hydroxy metabolites were found in studies using betamethasone or dexamethasone administration, however only the parent compound was detectable in triamcinolone studies.

References

The analysis of volatiles and semi-volatile samples in air on-site is important in light of the pertinent environmental and health hazards that may be present. Consequently, several sampling and analysis strategies have evolved to address these challenges. Sample pre-concentration followed by thermal desorption has been proven to increase the analysis dynamic range and to provide reliable and reproducible results (1).

One major difficulty encountered with the pre-concentration technique used in GC/MS systems is that transfer of the analyte to the column is often hindered by transfer lines or improperly matched flow rates. In addition, poor column retention for very light volatiles is often observed. These effects can lead to band broadening and poorly resolved or unresolved peaks. Cryofocusing can alleviate this problem for many compounds, but typically must be performed in a laboratory setting.

We have developed a split/splitless GC injector that has an incorporated thermal desorption capability complete with cryofocusing at the head of the column. This injector/desorber is built into a portable GC/MS system to facilitate on-site sampling. The injector/desorber system has a small localized cooling region inside the GC oven to cryofocus the more volatile compounds that might be found at a contaminated site.

Compounds that have a high vapor pressure at low temperature are particularly difficult to analyze properly and thus cryofocusing has to be well controlled. A temperature of at least -40°C is desirable. Benzene, for example, has a slight vapor pressure at temperatures of approximately -40°C and thus cryofocusing may be required. The results of concentrating a 10 ppbv sample of benzene for different periods of time followed by thermal desorption directly to the GC column are quite linear with precisions under 10%. For these experiments the GC column is sprayed with chlorodifluoromethane just prior to desorption. The resulting column temperature is approximately -50°C.

Chloroform has roughly a five times higher vapor pressure than benzene at -40°C and consequently it is even more difficult to generate a narrow GC band. The abundance versus concentration time profile for chloroform is shown in Figure 1. The GC/MS data for a 15 second concentration time from 50 ppbv is shown in Figure 2. The precision for this concentration time is approximately 15%.
Another compound of environmental interest that we have concentrated and desorbed is methyl isobutyl ketone (MIBK). An abundance versus concentration time profile is shown in Figure 3. The GC/MS data for a 15 second concentration time is shown in Figure 4. The precision of this measurement is 17%.

DETERMINATION OF POLAR VOLATILE ORGANIC COMPOUNDS
BY ATMOSPHERIC SAMPLING GLOW DISCHARGE/ION TRAP MASS SPECTROMETRY
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A glow discharge/ion trap mass spectrometer system has been evaluated for the direct atmospheric sampling and rapid measurement of trace levels of polar volatile organic compounds (VOCs) in air. These compounds are difficult to characterize using existing technology for nonpolar compounds (EPA's Method TO-14), largely because of the drying step normally used to remove co-collected water vapor from the sample and the need to preconcentrate the compounds to achieve the desired analytical sensitivity.

Interfacing the source with the quadrupole ion trap mass spectrometer offers a system with high sensitivity, simplicity, compact size, and tandem mass spectrometry (MS/MS) capabilities for compound characterization. The operating parameters which influence the ion injection efficiency in the ASGDI/ITMS are: low mass cut-off during ion injection, discharge current, buffer gas pressure in the ion trap, and focusing lens voltages. Figures 1(a)-1(d) show the results of varying these parameters while measuring the positive ion intensities for n-butylbenzene. Figure 1(b), for example, shows that the intensity of the fragment ion m/z 91 (C\textsubscript{T}H\textsubscript{8}) increases relative to m/z 92 (C\textsubscript{T}H\textsubscript{9}) due to an increase in fragmentation as the energy transferred to the molecular ion increases with increasing discharge current.

In the positive ion mode, the major reagent ions in the background air spectrum are NO\textsuperscript{+} (m/z 30) and O\textsuperscript{+} (m/z 32) and are essentially unaffected by the relative humidity (RH). These ions serve as reagent ions for the ionization of trace VOCs in air by electron or proton transfer. ASGDI spectra of aliphatic alcohols exhibit prominent peaks corresponding to either the (M+H\textsuperscript{+}) or (M-H\textsuperscript{-}) ion, or both. Both of the polar aromatics (benzaldehyde and nitrobenzene) and the nonpolar aromatics (benzene and toluene) produce large molecular ion peaks corresponding to (M+H\textsuperscript{+}), (M-H\textsuperscript{-}) or (M+H\textsuperscript{+}) in the ASGDI/ITMS system. Major peaks are observed in the ASGDI spectra at (M+H\textsuperscript{+}) for acetone and at M\textsuperscript{-} for methyl ethyl- and methyl isobutyl ketone. Results for the alkyl thiols studied are presented in Figure 2. In both of the nitriles examined (Figure 3), the base peak corresponds to the pseudomolecular ion at (M+H\textsuperscript{+}). Figure 4 shows that ethyl acrylate and methyl methacrylate give intense peaks for the (M+H\textsuperscript{+}) ion as well as for the fragment containing the carbonyl group (m/z 55 in ethyl acrylate and m/z 67 in methyl methacrylate). The results obtained in the present study for the nonpolar halocarbons, dichloromethane, chloroform, carbon tetrachloride, methyl chloroform, trichloroethylene, and tetrachloroethylene show generally good agreement with EI spectra and with the data of Asano, et al.

In the negative ion mode, the relative abundances and absolute intensities of the reagent anions are strongly dependent on the relative humidity of the background air. In relatively dry air (11% RH), the spectrum is dominated by the NO\textsuperscript{-} ion. However, in humid air (40% RH), the major peaks correspond to O\textsuperscript{-} and NO\textsuperscript{-}, with small contributions from O\textsuperscript{2-} and NO\textsuperscript{3-}. The spectra of the aliphatic alcohols obtained at 40% RH (e.g. Figure 5) show a prominent peak at (M-3)\textsuperscript{-}. It is assumed that O\textsuperscript{-} reacts with the alcohols to form the (M-3)\textsuperscript{-} ion by the initial loss of one proton to form the alkoxide anion, followed by the loss of an H atom from both the α- and the β-positions with respect to the OH group. The mass spectra for these alcohols at 11% RH show a (M+43)\textsuperscript{-} due to the addition of the m/z 46 (NO\textsubscript{2})\textsuperscript{-} and the loss of three H atoms. The most intense peak for both the aliphatic ketones and aliphatic carboxylic acids [Figure 6(a)] occurs at (M-H\textsuperscript{-}) in humidified air. Figure 6(b) shows the selectivity obtained for a mixture of aliphatic acids by negative ion ASGDI. The polar aromatic compound nitrobenzene displays the base peak at M\textsuperscript{-} and an intense peak at (M-H\textsuperscript{-}) in dry air, while benzaldehyde shows the base peak at (M+O-H\textsuperscript{-}). All of the alkyl thiols are characterized by intense peaks at m/z 64 (S\textsubscript{2}) and 80 (S\textsubscript{2}O). The two acrylic esters studied (ethyl acrylate and methyl methacrylate) give intense peaks for the molecular ion M\textsuperscript{-} and m/z 46.

Although polar VOCs give rise to mixed EI and CI ASGDI spectra in the positive ion mode, the fragment ion abundances are unlike their standard EI counterparts. Strategies to reduce the degree of fragmentation in the positive ion mode should be investigated.

References
Figure 1. Factors influencing the ion injection efficiency in the ASME/IMS

Figure 2. Positive ion ASME/IMS mass spectra of aliphatic thiols
(a) ethanethiol, (b) 1-propanethiol, (c) 2-propanethiol

Figure 3. Positive ion ASME/IMS mass spectra of aliphatic esters
(a) acrylic ester, (b) methyl methacrylate

Figure 4. Acetic acid (MW 60)

Figure 5. Negative ion ASME/IMS mass spectra of isopropyl alcohol

Figure 6. Negative ion ASME/IMS mass spectra of aliphatic acids.
DEMONSTRATION OF MOBILE MASS SPECTROMETRY THROUGH THE EPA SUPERFUND INNOVATIVE TECHNOLOGY EVALUATION PROGRAM

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The United States Environmental Protection Agency’s Office of Solid Waste and Emergency Response and Office of Research and Development established the Superfund Innovative Technology Evaluation (SITE) Program to promote the use of innovative technologies to clean up Superfund sites across the country. The SITE Measurement and Monitoring Technologies Program provides assistance in the development and demonstration of innovative technologies to characterize Superfund sites. Through EPA’s Environmental Monitoring Systems Laboratory in Las Vegas, Nevada (EMSL-LV) with the cooperation of Tufts University and Lockheed Engineering & Sciences Co., the technology of mobile mass spectrometry was demonstrated to be a promising technique for the characterization of pollutants at Superfund sites.

The goals of this demonstration were to evaluate the performance characteristics of the Mobile Environmental Mass Spectrometer (MEM) developed by Bruker Instruments Inc., (Billerica, Massachusetts) as a technique for on-site monitoring, and to generate substantial data with which to support the credibility of field measurements using a mass spectrometer.

Two active Superfund Sites in Massachusetts were selected as locations for this demonstration to take place. During August and September of 1990, the analysis of PCB contamination in soil samples took place at the N. Dartmouth, MA Superfund Site, while the analysis of PAH contamination in soil samples and VOC contamination in "spiked" water samples took place at the Westborough, MA Superfund Site. A range of sample preparation and analysis techniques were performed to rapidly generate qualitative and quantitative results on a real-time basis. The analytical plan consisted of 100% split sampling. All samples were analyzed on site using the MEM, with split samples shipped to a CLP laboratory for confirmatory analysis following the guidelines of a comparable EPA method of analysis. For both the PCB and PAH analytical events, the MEM was used to quickly screen a series of samples to establish a dynamic range of contamination. Upon selection of these samples as a result of screening, additional soil was collected, homogenized, and labeled as samples for analysis. The VOC analysis was performed on water samples taken from a pond located on site, due to the inability of accessing the monitoring wells. These water samples were spiked with known volatile organic contaminants at five different concentration levels. Along with quality control standards and the analysis of blanks, seven replicate samples were measured each day for a five day period with each of the three analytical events. Additionally, after the completion of sample analysis, a two week study was performed with the analysis of PCB and PAH Standard Reference Materials (SRM).
The sample preparation and analytical procedures used during the demonstration were unique in themselves. For PAH and PCB laden soil samples, the preparation involved the extraction of 0.5 grams soil with 2.0 mls organic solvent followed by a two minute hand shaking. An aliquote of the extract is injected onto the clean surface of a piece of aluminum foil. Bruker's patented Sampling Probe was used to thermally desorb the contents of the extract from the aluminum foil into a 3.5 M capillary sampling line yielding results in less than 7 minutes. The analysis of VOC contamination involved the adsorption of analytes onto adsorption tubes by a purge and trap technique with the aid of a pump. The equipment necessary for this technique in minimal consisting of a small battery powered low flow pump, tygon tubing, a sample impinger and an adsorbent tube. The tube was then placed into the desorption chamber of the mini-GC to give a full GC/MS analysis in 20 minutes. This demonstration used both of the two possible data systems to acquire the chromatographic information. The internal data system has a monitor, which controls the entire mass spectrometer functions and uses pre-programmed methods for compound monitoring. The auxiliary data station is a MS DOS 386 Compaq computer which is interfaced to the internal data system when full scan acquisition is desired. This configuration allows for full GC/MS/DS capabilities.

This demonstration evaluated the MEM, on its own merits, and by comparing its results to those of a conformational laboratory. Precision was determined by evaluating the percent relative standard deviation (%RSD) of replicate samples for each analytical event. The MEM results show an average of 20% RSD being achieved for the analytes of each analytical event. Accuracy of each analytical event was determined by evaluating the results of Standard Reference Material (SRM) standards analyzed with the MEM. Most PAH SRM compounds were determined to have a negative bias of greater than 30%. This bias was consistent with the confirmatory laboratory indicating the possibility of degradation of the SRM. PCB accuracy also showed a negative bias greater than 40% with results falling between those of two confirmatory laboratories. VOC accuracy ranged between -70 to +200. At low concentrations, the confirmatory laboratory are generally closer to the theoretical value than the MEM concentrations. At higher concentrations, the bias for the MEM samples is generally equivalent or less that those obtained by the confirmatory laboratory. The overall completeness by sample type and by compound exceeded 90% in all cases. (1)

The intricacies of this study involve two matrices, and three different compound classes. The quality assurance project plan was established to allow a data base from which statistical evaluations could be conducted resulting in an accurate assessment. By doing so, a comprehensive comparison between on-site measurements using the MEM and off-site measurements using stationary mass spectrometric systems was completed. As a result, field GC/MS has been shown to be capable of yielding rapid, accurate, and cost effective analyses.

REFERENCES

HELIUM-PURGED HOLLOW FIBER MEMBRANE FLOW CELL FOR MONITORING VOLATILE ORGANIC COMPOUNDS IN WATER

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text

INTRODUCTION

Semi-permeable membranes offer the most direct means of detecting and monitoring volatile organic compounds in water. Two configurations have been described using hollow fiber membranes directly coupled to mass spectrometers. In the "flow-over" configuration, the interior of one or more hollow fibers is exposed to ion source vacuum while aqueous sample flows over the exterior surface (1,2). In the "flow-through" configuration, aqueous sample is passed through the fiber interior while the exterior surface is exposed to ion source vacuum (3-6). Preliminary results have been shown using a modified flow-over configuration in which helium is purged through the fiber interior (7). This work describes the results obtained by incorporating a single helium-purged hollow fiber membrane into a flow cell for real-time measurement of regulated volatile organic compounds in municipal drinking water.

TECHNICAL APPROACH

A flow cell utilizing a single 9 cm long silicon rubber hollow fiber shown in Figure 1 was used for this work. Carbon tetrachloride, trichloroethene, benzene, 1,1-dichloroethene and vinyl chloride in methanol were injected into ambient temperature reagent water or drinking water obtained from a laboratory tap, allowing fortified concentrations significantly less than 1 \(\mu\text{g}/\text{L}\) (PPB). The mass spectrometer was a Finnigan MAT TSQ-45 operating as a single analyzer instrument or a Finnigan MAT Model 700 ion trap (ITD). All measurements were made using electron ionization full scan conditions.

Extracted ion current profiles for three of the analytes are shown in Figure 2 for intermittent fortifications in municipal drinking water conducted with the TSQ-45 and a helium purge rate of 1.0 cc-atm/min. The response offset for carbon tetrachloride is due to chloroform present in the drinking water sampling stream. Response was linear for all analytes from 0.18 to 50 PPB except trichloroethene which was not observed at fortified concentrations less than 0.40 PPB.

Results using the ITD before and after 5 PPB fortification of a reagent water sampling stream revealed good signal-to-noise response for each analyte. A sequence of low level challenges were conducted with the ITD similar to those with the TSQ-45, but at lower fortified concentrations. Response was observed for benzene and vinyl chloride at concentrations as low as 0.09 PPB. Minimum detection levels achieved in this work and current Safe Water Drinking Act limits are compared in Table I.

CONCLUSIONS

The helium-purged hollow fiber configuration provides real-time full scan MS measurements of volatile organic compounds in drinking water at concentrations significantly below regulatory limits. The helium purge allows the use of relatively long hollow fibers for high sensitivity, and long transfer lines to the ion source. High sensitivity in real-time is important for process control applications to allow monitoring of trends and implementation of corrective action before regulatory limits are exceeded.
LITERATURE CITED


Figure 1

TABLE I
MAXIMUM CONTAMINANT LEVELS (MCLs)
FOR SELECTED COMPOUNDS IN DRINKING WATER

<table>
<thead>
<tr>
<th>Compound</th>
<th>Regulatory Limit, µg/L</th>
<th>Hollow Fiber Sensitivity, µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon Tetrachloride</td>
<td>5.0</td>
<td>0.18</td>
</tr>
<tr>
<td>Trichloroethene</td>
<td>5.0</td>
<td>0.40</td>
</tr>
<tr>
<td>Benzene</td>
<td>5.0</td>
<td>0.09</td>
</tr>
<tr>
<td>1,1-Dichloroethene</td>
<td>7.0</td>
<td>0.18</td>
</tr>
<tr>
<td>Vinyl Chloride</td>
<td>2.0</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Figure 2

668
Detection of Carbonyl Compounds in Drinking Water Using a Membrane Introduction Probe in a Tandem Mass Spectrometer.

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Because formaldehyde is known to be carcinogenic, there is a concern over possible health hazards from long time exposure to low concentrations of formaldehyde. Formaldehyde and other carbonyl compounds are formed as by-products during the ozonation of drinking water. These carbonyl compounds react with O-(2,3,4,5,6-pentafluorobenzyl)-hydroxyamine (PFBOA) in aqueous solution to form the corresponding oximes (eq 1) which are volatile, can pass through a semipermeable membrane, and can be detected by electron capture mass spectrometry.

\[
\begin{align*}
R_1 \quad &\quad C=O \\
R_2 \quad &\quad C_6F_5-CH_2-O-N=C < \quad R_1 \\
R_2 \quad &\quad C_6F_5-CH_2-O-NH_2
\end{align*}
\]

The objective of the present study was to detect low molecular weight carbonyl compounds at very low concentrations in aqueous solution by using a direct insertion membrane probe in a tandem mass spectrometer.

Figure 1 shows the experimental set up for the membrane introduction mass spectrometer developed in our laboratory and already described elsewhere (1,2). Figure 2 shows the negative ion chemical ionization mass spectrum of a mixture of PFBOA derivatives of formaldehyde, acetaldehyde and propanaldehyde. The following peaks in the mass spectrum provide evidence for the passage of the derivatives through the membrane.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mass (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCHO</td>
<td>225</td>
</tr>
<tr>
<td>CH₃CHO</td>
<td>239</td>
</tr>
<tr>
<td>CH₃CH₂CHO</td>
<td>253</td>
</tr>
</tbody>
</table>

The peaks at m/z 197, 181 and 178 are due to C₆F₅CH₂O-, C₆F₅CH₂N°C and (M' - HF - HCN (RCN)) respectively. The product (MS/MS) spectra of individual compounds shown in Figures 3, 4 and 5 confirm the identity of the characteristic peaks in the mixture. Detection limit for each of the aldehydes in the mixture was established by multiple ion monitoring during MS/MS experiments. The M' ions of PFBOA derivatives of aldehydes were selected with quadrupole one and the product ions m/z 205, 178, 181, m/z 219, 181, 178 and m/z 233, 178, 181 were monitored for the detection limit of formaldehyde, acetaldehyde and propanaldehyde respectively.

The detection limit of formaldehyde was found to be 1 ppb. That of acetaldehyde and propanaldehyde was found to be 10 ppb. In the case of formaldehyde, the range of linearity (shown in Figure 6) was between 1 ppb and 1000 ppb. Experiments are in progress for the establishment of the linear dynamic range of detection for acetaldehyde and propanaldehyde.

References:
Fig. 1. Schematic presentation of the membrane introduction apparatus used in this study.

Fig. 2. NCI Mass spectrum of PFBA derivatives of formaldehyde, acetaldehyde and propionaldehyde.

Fig. 3. Product Ion MS/MS spectrum of PFBA derivative of formaldehyde. Collision energy is 20 eV and collision gas (argon) pressure is 1 mtorr.

Fig. 4. Product Ion MS/MS spectrum of PFBA derivative of acetaldehyde. Collision energy is 20 eV and collision gas (argon) pressure is 1 mtorr.

Fig. 5. Product Ion MS/MS spectrum of PFBA derivative of propionaldehyde. Collision energy is 20 eV and collision gas (argon) pressure is 1 mtorr.

Fig. 6. Linear Dynamic range for formaldehyde.
REMOTELEY OPERATED, FIELD PORTABLE GAS CHROMATOGRAPHY/MASS SPECTROMETRY SYSTEM FOR MONITORING HAZARDOUS ATMOSPHERE VAPORS

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In order to protect and inform personnel involved in monitoring, containment and remediation of hazardous volatiles materials, it is important that exposure to such materials be limited and that personnel working in such environments have sensitive and easily handled tools which do not limit mobility or vision. In order to meet these requirements a man-portable GC/MS system described previously [1] has been modified to meet four objectives: (1) to allow remote instrument operation via a serial data transfer protocol compatible with broadband radio telemetry; (2) to reduce system size for placement in a single backpack or in a small, unmanned reconnaissance plane (drone); (3) to reduce system weight under 50 lbs for worker mobility in man-portable mode and to meet payload requirements for drone aircraft; (4) to increase pumping speed for greater sample throughput and lower detection limits.

Objective 1 has been met by replacing the on-board 386 computer data system with a National Instruments GPIB-110-2 unit integrated into the system. This converts the HPIB data base signals from the MSD (Hewlett Packard) data acquisition electronics to a synchronous, serial data transfer protocol. A second GPIB-110-2, up to 500 m away via coaxial cable, converts data back to GPIB signals at the remote computer data station. This system should be compatible with broadband telemetry for even longer remote transmissions.

Objectives 2 and 3 were partially met when the data system was removed from the portable MS chassis. The previous molecular drag pumping system (MDP 5010, Alcatel) was both slow (8 l/s, N2) and required a bulky (5 l) evacuated cylinder to provide only 2 hrs of backing pressure (@ .1 ml/min He). Clearly, a reduction in volume of this pumping system along with an increase in pumping speed would be necessary to meet criteria 2, 3 and 4.

Table 1 shows a comparison of various vacuum pumping technologies. The pumping system must be able to remove gas volumes greater than .1 ml/min to offer significant improvement in detection limits without sacrificing the preseparation power of the current "transfer line" GC sampling method [1,2]. While none of the off-the-shelf systems met all the needed requirements, it was clear that a potentially powerful system may be possible via a "chemical" pumping method if alternative carrier gases could be used and argon loading could be controlled.

Figure 1 is a drawing of a hybrid pumping system utilizing a modified bulk getter pump (Danielson) designed to minimize heat loss to the environment via a low emissivity Ni getter crucible and to pump background Ar from atmospheric sampling via a 2 l/s ion getter pump (Perkin Elmer). This pumping system operates on 10 W, weighs approximately 10 lbs and pumps more than 50 l/s H2 or 15 l/s N2.

Effective utilization of this system requires compatibility of alternative, non-noble carrier gases with the MSD analyzer system. Figure 2 shows a comparison of MSD operation for the analysis of trace toluene levels in air. At similar pressures, baseline noise levels for all detected ions increased with increasing carrier gas molecular weight. (Presumably due to improved momentum transfer between carrier neutrals and ions in the analyzer). Further, the lower viscosity of H2 caused a significantly higher analyzer pressure and shorter retention time using the same capillary column. The high noise level at .3 ml/min flow rate discouraged N2 usage, but it appears that with a slightly longer column H2 is an effective carrier which is easily pumped by bulk and ion getter systems.

The present system is seen in the block diagram and profile drawings of Figure 3. Because of the low weight and size of this new pumping system, we have met objectives 2 and 3. At this time we are still verifying expected upper ppb detection limits (in SIM mode) utilizing this new pump and H2 carrier.


ACKNOWLEDGEMENTS

This work was sponsored by the U.S. Army, CRDEC and the Advanced Combustion Engineering Research Center. Funds for this center are received from the National Science Foundation, the State of Utah, 23 industrial participants and the U.S. Department of Energy. The authors wish to express their gratitude for the help and advice of Jean Luc Truche and John Fieldsted (Hewlett Packard Corporation).
Table 1 - Comparison of Tested Vacuum Technologies

<table>
<thead>
<tr>
<th>Technology</th>
<th>Backing Pressure</th>
<th>Noble Gas Speed</th>
<th>Power</th>
<th>Lifetime</th>
<th>Weight</th>
<th>Mobile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusion Pump (Edwards)</td>
<td>&lt;10^-9 torr</td>
<td>&gt;60%</td>
<td>&gt;200 W</td>
<td>&gt;6 mo</td>
<td>40 lbs</td>
<td>no</td>
</tr>
<tr>
<td>Turbomolecular (Alcatel)</td>
<td>&lt;10^-9 torr</td>
<td>&gt;60%</td>
<td>&gt;200 W</td>
<td>&gt;6 mo</td>
<td>40 lbs</td>
<td>no²</td>
</tr>
<tr>
<td>Molecular Drag* (Alcatel)</td>
<td>&lt;10^-9 torr</td>
<td>&gt;60%</td>
<td>&gt;20 W</td>
<td>&gt;2 hrs</td>
<td>20 lbs</td>
<td>no²</td>
</tr>
<tr>
<td>Ion Getter (Varton)</td>
<td>None</td>
<td>30% max</td>
<td>150 W</td>
<td>-1000 hrs</td>
<td>25 lbs</td>
<td>yes</td>
</tr>
<tr>
<td>Bulk Getter (Danielson)</td>
<td>None</td>
<td>0</td>
<td>40W</td>
<td>1 week</td>
<td>15 lbs</td>
<td>yes</td>
</tr>
</tbody>
</table>

Includes mechanical backing pump
1 highly pressure dependent (6.3 x 10^-9 torr)
2 using 4 1 evacuated reservoir for roughing
3 Alcatel models will tolerate vibration and multiple
4 Actual model tested was 8 lbs, 30 lbs model requires H2O cooling, sweep gas and 80 W.

Figure 1. Schematic of Bulk/Ion Getter pumping system.

Figure 2. Selected ion chromatograms, m/z 91, for analysis of toluene in air (4 repetitive analyses are shown in each trace). (a) 1.3 ppm with Ar carrier gas. The extremely high noise level completely obscures the toluene peak at .1 min after the air pulse. (b) 1.3 ppm N2 carrier gas yields lower noise than the Ar trace. (c) Approximately 250 ppb with He carrier gas yields the lowest overall noise level, but H2 should be comparable at similar pressures. (d) Approximately 250 ppb with H2 carrier gas. Source pressure is 2 X 10^-9 torr, 2.5 times the pressure for the other three gases, and the retention time is shifted to .05 min due to lower H2 viscosity.

Figure 3 - (a) block diagram of portable GC/MS system units. (b) schematic showing system chassis for the portable GC/MS system.
A HIGH-SPEED, HIGH-GAIN PRECONCENTRATOR FOR MASS SPECTROMETERS: APPLICATIONS TO EI, CI, AND DISCHARGE SOURCES

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This paper describes a high-speed, high-gain preconcentrator that can dramatically improve the real-time air-monitoring capabilities of mass spectrometers. Conventional preconcentrators produce high sensitivity to trace-level constituents at the expense of long sampling times. The preconcentrator described here uses low-pressure and low-flow-rate desorption conditions to increase the concentration of the desorbed analyte in the carrier gas. As a result, it can provide high sensitivity with short sampling times. This device was tested with both EI and CI ion sources. Using 3-second sampling times, the preconcentrator enhanced EI signals by over 500-fold compared with direct sample introduction. The preconcentrator also improved the CI signal 18-fold.

A preconcentration factor or "gain" can be defined as the ratio of the fractional molecular concentration $f_d$ of a trace analyte in the desorption carrier gas to its concentration $f_s$ in the sample gas ($G = f_d / f_s$). The gain can also be expressed in terms of the adsorption and desorption flow conditions by noting that the number of analyte molecules desorbed from an ideal preconcentrator equals the number of analyte molecules adsorbed from the sample gas. The number $N$ of analyte molecules adsorbed on the sorbent is

$$N = f_s n_s W_s t_s$$  \hspace{1cm} (1)

where $n_s$ and $W_s$ are the number density (in cm$^{-3}$) and volume flow rate (cm$^3$ s$^{-1}$) of the sample gas entering the preconcentrator and $t_s$ is the sampling time. The number of analyte molecules desorbed from the sorbent is

$$N = f_d n_d W_d t_d$$  \hspace{1cm} (2)

where $n_d$ and $W_d$ refer to the carrier gas leaving the preconcentrator during the time $t_d$ required for desorption. Combining Eqs. (1) and (2) yields an expression for the preconcentration gain $G$

$$G = \frac{f_d}{f_s} = \frac{n_s}{n_d} \frac{W_s}{W_d} \frac{t_s}{t_d} = \frac{Q_s}{Q_d} \frac{t_s}{t_d}$$  \hspace{1cm} (3)

where $Q$, the mass flow rate in units of molecules per second, is the product of the number density and the volume flow rate.

Eq. (3) shows that the preconcentration gain factor is proportional to gas flow rate during the sampling period and the sampling time, as is commonly recognized. Eq. (3) also illustrates the importance of the desorption conditions.

In viscous flow, the mass flow rate $Q$ through the sorbent is proportional to the product of the average pressure in the sorbent and the pressure difference across the sorbent. Therefore, we can write

$$G = \frac{f_d}{f_s} = \frac{Q_s}{Q_d} \frac{t_s}{t_d} = \frac{<p>}{<p_d>} \frac{\Delta P_s}{\Delta P_d} \frac{t_s}{t_d}$$  \hspace{1cm} (4)

where $\Delta P = (p_f - p_0)$ is the pressure difference between the ends of the sorbent tube and $<p> = (p_0 + p_f)/2$ is the average pressure in the tube.
Eq. (4) shows that reducing both the pressure of the desorption gas and the pressure drop across the sorbent tube leads to enhancements in the concentration of the analyte in the carrier gas. The high-speed, high-gain preconcentrator described here uses low-pressure and low-flow-rate desorption conditions to produce high concentration gains without long sampling times.

This preconcentration concept was tested with a mass spectrometer equipped with an electron-impact ionization source. Gains of greater than 500 were observed with approximately equal sampling and desorption times (3 seconds). The mass flow rate during sampling Q_s was approximately 150 sccm from an atmospheric pressure sample (p_s = 370 torr and Δp_s = 670 torr), while the desorption mass flow rate Q_d was approximately 0.2 sccm (p_d = 19 torr and Δp_d = 21 torr). The gain was measured by comparing the ion signal produced by the preconcentrator and the signal generated from direct introduction of the sample at the same pressure in the ion source (2 x 10^-5 torr). The total preconcentrator cycle time, including the time required to cool the sorbent, was less than 35 seconds.

The same preconcentrator was applied to chemical ionization source using 3-second sampling and desorption times. This configuration produced gains of approximately 18 compared with direct sample introduction at the same source pressure. The difference in gain between the CI and EI sources results from the difference in desorption flow rates required by the two sources. As noted above, the EI source operated with desorption mass flow rates Q_d of about 0.2 sccm, while mass flow rates Q_d of about 3.7 sccm (p_d = 70 torr and Δp_d = 100 torr) were needed to produce high enough pressures in the ion source (approximately 0.5 torr) for effective chemical ionization.

Reducing the gas throughput of CI sources and low-pressure discharge sources, by reducing their leak rates, can reduce the required desorption gas flow rate Q_d and produce higher preconcentration gains. However, the residence time of the analyte gas in the source, which is determined by the ratio of the source volume and the volume flow rate, must be kept small compared to the desorption time. Otherwise, the advantages of rapid desorption times are lost. This limitation requires careful design of these sources to fully exploit this new low-pressure, low-flow-rate preconcentrator.

We expect that this preconcentrator will enable mass spectrometers with traditional electron-impact ionization sources to detect contaminants at concentrations below 1 part per billion in real time. With proper design of the CI and discharge sources, this preconcentration approach may push the real-time detection limits of these ionization sources into the part per quadrillion range.

This work was supported by the Office of the Program Manager for Chemical Demilitarization under contract DAAA-15-B-0107 and the National Institute of Environmental Health Sciences under contract 1R43ES05151-01.

The 39th ASMS Conference on Mass Spectrometry and Allied Topics
Evaluation of a Time-of-Flight Mass Spectrometer (TOF-MS) as a Multi-purpose Detector for Environmental Monitoring

Paul Farrow, James Kyranos and Peter Ralbovsky, Jim Valentine
Arthur D. Little, Inc., Cambridge, MA 02140.

Continuous monitoring of potentially hazardous compounds in the environment has become increasingly important in the last decade. In some cases rapid on-site analysis is a viable alternative by the use of "mobile" laboratories. However for some applications and circumstances, to send out a mobile laboratory is impractical, e.g. space station, submarines, rocket launch pads. Conventional GC-MS systems can be expensive and difficult to maintain over extended periods of time (several years of continuous operation is often necessary). A potential solution is the development of a rugged, durable, multi-purpose detector for permanent monitoring. The development of a TOF-MS has been considered for this application.

A standard CVC (CVC Rochester, NY) MA-3 TOF was used for this development program to investigate the feasibility of TOF-MS. The inlet system was from Brunfeldt Company (Bartlesville, TX) and modified for our application. A strip chart recorder and PC-based data system were used to collect data (data acquisition system developed at Arthur D. Little).

Two aspects of the design are discussed in this paper along with data collected from the development instrument:

- Effect of low ionization energy on spectra
- Resolution of Instrument related to acquisition rate

Effect of low ionization energy on spectra

Determining the presence of low molecular weight volatile species in the presence of an air background were investigated without the use of chromatographic separation. By the use of low ionization energies (10-15eV), we were able to eliminate oxygen and nitrogen backgrounds from the spectra.

Hydrazine, an analyte of interest and the same molecular weight as nitrogen was introduced directly into the MS source. The spectra taken at 70eV (Figure 1), shows contribution from air masking the hydrazine. However, by dropping the ionization energy to 10eV, we were able to eliminate the air, and obtain a representation spectra of hydrazine (Figure 2) that can be used for qualitative determination.

Resolution of Instrument related to acquisition rate

The design of a data acquisition system is controlled by the amount of power available, physical size and application. As a result of understanding the application, the rate of data acquisition can be greatly reduced, thus reducing the complexity of the hardware involved. For this particular system, we investigated the trade-off between acquisition "bin size" and mass resolution. Figure 3 shows that at a 10ns "bin size," m/z 28 and 32 are well resolved. Even at 200ns, there is sufficient resolution between the two masses. The saving in power by using 200ns versus 10ns is very important when considering space station applications, where power is very limited.

To date, the TOF-MS has demonstrated adequate sensitivity and selectivity for certain analytes of interest. The data system requirements for the acquisition rate will be dependent on the specific application and the need to have unit mass resolution or not. From the data collected on hydrazines, it is apparent that operating at low ionization energies, thus eliminating the air background, a decreased resolution will be sufficient.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Analyte: Hydrazine
Pressure: 8.1x10^{-6} Torr.
Ionization Energy: 70.22 eV
Recorder Range: 5 V

Equivalent graph for Analyte: Hydrazine
Pressure: 8.5x10^{-6} Torr.
Ionization Energy: 10.02 eV
Recorder Range: 50 mV

FIGURE 1
THE EFFECT OF BIN SIZE VERSE PEAK RESOLUTION

FIGURE 3

We are acknowledging partial support of this work under a subcontract with Lockheed Missiles & Space Company, Inc., subcontract number HS80E3430N.
USE OF A TAGA 6000E MS/MS ON AN AIRBORNE MONITORING PLATFORM TO MEASURE SPECIES OF INTEREST IN GLOBAL CLIMATE CHANGE

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Columbus, Ohio 43201-2693

INTRODUCTION

The increased interest in conditions affecting global climate change has made it necessary to incorporate airborne monitoring platforms to obtain data aloft. Most instruments carried aloft can obtain real-time data, but only on a limited number of species (e.g., NO, NO\textsubscript{2}, \textit{O}_3, etc.). To obtain data on more complex species, air samples have been collected in canisters or on sorbent media and analyzed later. These limitations inhibit the assessment of 3-dimensional spatial variations in atmospheric concentrations for complex compounds of interest. To obtain spatial concentration data on more complex species in real-time, a feasibility study was conducted to evaluate the potential of atmospheric pressure chemical ionization (APCI) tandem mass spectrometry for continuous monitoring of compounds of interest in global climate change.

TECHNICAL APPROACH

A Sciex TAGA 6000E tandem mass spectrometer with an APCI source was used for this feasibility study. The TAGA was installed in Battelle's Douglas DC-3 aircraft for these flights. Minor modifications were made to the aircraft to supply power to the TAGA, as well as to shock mount the instrument to the floor of the aircraft. These were the only modifications that were required. Sample air was provided from a positive pressure sampling port located near the front of the aircraft ahead of the engine exhaust. A split in the sample line allowed control of the air flow to the TAGA.

Test flights were made in the central Ohio area by slowly spiralling down from 10,000 feet to 2,400 feet. This altitude window allowed us to sample above, as well as within the mixing layer of the atmosphere. Compounds continuously monitored were acetone, formic acid, acetic acid, peroxycetyl nitrate (PAN), NO\textsubscript{2}, and SO\textsubscript{2}. Multiple reaction monitoring was performed in positive or negative ion modes to maximize sensitivity for the compounds of interest.

In addition to the vertical profile experiments, monitoring was conducted while circling a trash burning power plant to identify plume boundaries and compounds in the plume.

RESULTS

Figures 1 and 2 are examples of the vertical profiles for acetone and SO\textsubscript{2}. Acetone concentrations ranged from 0.5 ppbv to 2.75 ppbv, formic acid concentrations ranged from 1.0 ppbv to 5.0 ppbv, acetic acid concentrations ranged from 0.2 ppbv to 0.7 ppbv, PAN concentrations ranged from 0.1 ppbv to 0.5 ppbv, NO\textsubscript{2} concentrations ranged from 5.0 ppbv to 13 ppbv, and SO\textsubscript{2} concentrations ranged from 2.5 ppbv to 28 ppbv. Concentration ranges for all of the species monitored are typical of the atmospheric concentrations in the central Ohio area.
CONCLUSIONS

We have successfully demonstrated the capability of real-time MS/MS monitoring of atmospheric species on an airborne monitoring platform. This capability opens new techniques that the atmospheric scientist can utilize in the study of global climate change, model verification, and pollution detection and quantification. Future experiments are scheduled to monitor species along the eastern seaboard between Washington, DC and Cape Hatteras, NC in the fall of 1991.

Figure 1

VERTICAL ACETONE PROFILE

Figure 2

VERTICAL SO2 PROFILE
Identification of a Corrosive Agent in an Industrial Process:
Mass Spectrometry As Part of a Multi-Technique Problem Solving Effort

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During the operation of a process column, catastrophic corrosion began to occur in the reboiler. This system which involved an aqueous methyl diethanolamine (MDEA) solution did not typically give corrosion rates even approaching the several hundred mils per year rate observed in this system. The rate of corrosion grew as the process solution aged, suggesting that some reaction product of the amine or other substance which built up in the system was to blame for the corrosion. Deposits of black material were found in the system which were identified by X-ray diffraction and Energy Dispersive X-ray Fluorescence as iron sulfide. NMR and FTIR analysis revealed traces of materials not found in virgin amine solution which could be oxidation products of the amine, but no specific identification could be made with these techniques. Analyses typically used to evaluate the amine solution in the production plant did not show any abnormal results which could account for the corrosivity. Cyclic voltammetry helped characterize the corrosion, but without a suspect corrosive agent, could not provide an identification of the culprit.

The literature suggested that a three step mechanism might be active in the corrosive process. The first step involved acidic attack on the materials of construction, carbon steel in this case. Acids are certainly present in the system in the form of so-called heat stable salts of the amine. Various acids such as formic, acetic and thiocyanic are known to be present and form salts with the amine. The concentration of these salts tends to increase because heat does not regenerate the amine from them. The second step is said to involve chelation of the dissolved iron so that it can be transported to a region where the third step becomes operative. In the third step, the iron is precipitated by sulfide freeing the chelating agent to tie up and transport more iron so that it can also be precipitated. Iron sulfide was already shown to be forming in the system in copious amounts. Thus, the corrosive agent might be some chelating agent unique to this process which is as yet unidentified.

In order to obtain a more specific identification of the corrosive agent GC/MS analyses were conducted. A number of materials were identified. Figure 1 shows a typical result. Some substances which were thought to be possible corrosive agents were not found either in the GC/MS runs or direct-probe MS runs. One relatively large impurity peak was observed by GC/MS and remained to be identified. (Figure 1 "145 m/z") As will be seen this material proved to be the chelating agent suggested by the three step mechanism.
EI-MS (Hewlett-Packard 5985B GC/MS/DS) indicated that the compound potentially had molecular weight of 145 Da, contained nitrogen, and probably a primary alcohol, similar to MDEA. Isobutane CI confirmed the 145 Da molecular weight. GC/FTIR/MS (Hewlett-Packard 5890A GC/5965A FTIR/5970B MS) indicated that the compound had a carbonyl band at 1781 cm⁻¹. This suggested that the compound was a carboxylic acid. Accurate mass measurements (VG 70-SE) showed that the compound had the empirical formula C₆H₁₁NO₃. GC/MS/MS (Finnigan TSQ-70) experiments showed that the fragmentation sequence was unremarkable being sequentially stepwise down from m/z 145. (145-114-86-56-42) The presence of six carbon atoms in the compound was surprising in that MDEA has only five carbons.

Brainstorming of possible structures suggested that a lactone containing nitrogen might be possible. Lactones and carboxylic acids both turn out to have carbonyl bands near 1780 cm⁻¹ in the vapor-phase. The specific compound 4-hydroxyethyl morpholin-2-one can be envisioned to form by cyclodehydration of N,N-bis-hydroxyethyl glycine, otherwise known as bicine. Bicine is very similar to compounds expected from oxidation of the MDEA except for the extra carbon. The literature indicated that bicine is a strong chelator of ferric ion. Evidently, the bicine was reacting in the GC injector to produce the lactone. (Figure 2) Synthesis of bicine revealed that it gives the lactone seen in the GC analysis. The lactone so produced has the same EI spectrum as the unknown. Direct-probe MS analysis of bicine gave spectra that were mixtures of spectra of the lactone and bicine.

The origin of bicine was the next question. Two mechanisms have been proposed as possible explanations for the formation of bicine from MDEA. The first involves disproportionation of the ammonium ion of MDEA to give various mixed amines including N-methyl ethanolamine, N,N-dimethyl ethanolamine, diethanolamine and triethanolamine. Triethanolamine was shown to oxidize in air to bicine. The other mechanism uses formaldehyde and cyanide ion both present in the system to produce the nitrile analog of bicine which is hydrolyzed to bicine.

The identification of bicine as the cause for the corrosion has led to a number of approaches for eliminating the problem. Several of these are being investigated presently.

![FIGURE 1](image1.png)

![FIGURE 2](image2.png)
GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) FOR REACTIVE ORGANIC COMPOUNDS

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INTRODUCTION

Reactive chemical compounds present many problems for analysis by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). These problems extend from the failure to detect these compounds at low levels to rapid decrease of the response factors, which are used for quantitation, of these compounds with time. Quantitative methods such as EPA Methods 525 and 625 require very precise reproducibility of response factors for many very reactive compounds at low concentration levels. Most GC/MS systems presently used for analysis of these methods can only meet the QC/QA requirements for a short time, especially for Method 525. An enhanced GC/MS system has been developed at the Oak Ridge Y-12 Plant. The system allows for the analysis of reactive compounds like those of EPA Method 525 and 625 at concentration levels much less than those required by Method 525.

EXPERIMENTAL

The experiments were performed on a Kratos MS-50 mass spectrometer equipped with the Enhanced GC/MS System. A Restek Rtx-5, 30 meter, 0.32 mm ID, 1.0 μm df column was used. The mass spectrometer was scanned at 0.5 sec/dec from 35-600 amu.

RESULTS AND CONCLUSIONS

Figure 1 shows the results of a 1 ng per component injection on the system after the system had been in maintenance-free service for 1 year. The results show excellent sensitivity of the Enhanced System for the highly reactive phenols of EPA Methods 525 and 625 when compared to the internal standards that are also injected at a 1 ng concentration level. The internal standards used were 1,4-dichlorobenzene-D4, naphthalene-D8, acenaphthene-D10, phenanthrene-D10. All of the phenols have excellent peak shapes. Table 1 shows the effect on performance of a year's operation on the Enhanced System. Even after this time, the data show no reduction in response to the phenols. The May 1990 data represent the average over 14 days, while the April 1991 data represent a single analysis. The Enhanced System has proven to prevent column bleed from reaching the mass spectrometer ion source. This reduction of bleed is so great that no background subtraction is required, even when 100 pg levels of the phenols are analyzed. The Enhanced System has proven its ability to greatly improve identification of compounds with very low response factors at concentration levels of less than 100 pg. The system has been able to get perfect identification on compounds, such as pentachlorophenol and 2,4-dinitrophenol at a concentration less than 100 pg.

Table 1. Change in response factors for phenols after one year

<table>
<thead>
<tr>
<th>Compound</th>
<th>May 1990</th>
<th>April 1991</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>0.66</td>
<td>0.64</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>0.19</td>
<td>0.17</td>
</tr>
<tr>
<td>2-Nitrophenol</td>
<td>0.59</td>
<td>0.88</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>0.13</td>
<td>0.18</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>0.63</td>
<td>0.55</td>
</tr>
<tr>
<td>4-Chloro-3-Methylphenol</td>
<td>0.43</td>
<td>0.36</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>1.36</td>
<td>1.33</td>
</tr>
<tr>
<td>4-Nitrophenol</td>
<td>0.75</td>
<td>0.50</td>
</tr>
<tr>
<td>2-Methyl-4,6-Dinitrophenol</td>
<td>0.56</td>
<td>0.49</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>0.50</td>
<td>0.54</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>0.81</td>
<td>0.68</td>
</tr>
</tbody>
</table>

1 mg of each compound injected.

Average of four analyses.

Single analysis.

DS98 Chromatogram report Run: TEST0015, 3-Apr-91 14:30
TEST 1NG PHENOLS

1) Phenol
2) 2-Chlorophenol
3) 2-Nitrophenol
4) 2,4-Dimethylphenol
5) 2,4-Dichlorophenol
6) 4-Chloro-3-Methylphenol
7) 2,4,6-Trichlorophenol
8) 2,4-Dinitrophenol
9) 4-Nitrophenol
10) 2-Methyl-4,6-Dinitrophenol
11) Pentachlorophenol

Scan 580 1080
R.T. 13:52 18:47

682
A COMPARISON OF VARIOUS GC/MS INTERFACES FOR THE ANALYSIS OF VOLATILE ORGANIC COMPOUNDS

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EPA Method 524.2 is the capillary gas chromatography/mass spectrometry (GC/MS) method for the analysis of volatile organic compounds in drinking water. This method specifies full scan data acquisition and special MS tuning to meet p-bromofluorobenzene (BFB) ion ratio criteria. Because this method requires much lower detection limits than the "600 series" counterpart (EPA Method 624), the HP 5971A benchtop mass spectrometer and MS Engine were evaluated for the low-level volatile organic analysis (VOA) in drinking water.

Method 524.2 allowed for the use of 0.75, 0.53 (known as megabore) and 0.32 mm inner diameter (ID) columns. This study was conducted to evaluate different instrumental configurations necessary to implement 0.53 mm and 0.32 mm ID columns in both singly and differentially pumped mass spectrometers. Three MS configurations: megabore/jet, megabore direct and capillary direct were used. The HP 5971A Mass Selective Detector (MSD) required a jet separator to compensate for the high flow rates of the 0.53 mm ID column (8-15 ml/min). With the differentially pump MS Engine, the 0.53 mm ID column was interfaced directly. The 0.32 mm ID column was interfaced directly to the HP 5971A source but required cryofocussing. The MSD results obtained from the 0.53 mm ID column with a jet and the 0.32 mm ID column with a capillary direct interface were compared.

Figure 1 shows chromatograms from each of the three configurations. Twenty-five milliliter samples were analyzed in each configuration. Because of the large amount of CO\textsubscript{2} transferred from the trap to the column (from the charcoal), each chromatogram was either background subtracted or the chromatogram from mass 48-260 amu extracted. Comparing the HP 5971A with the 75 m, 0.53 mm ID column and the 50 m, 0.32 mm ID column the run times are similar. The absolute counts (y-axis) for the capillary direct configuration are about two times higher showing better transfer of sample from the purge and trap to the mass spectrometer.

The results of this study showed that the longer 0.53 mm ID columns (>50 m) eliminated the need for cryogen. The GC oven could be started at 35 °C and the light gases would still be chromatographed. Cryogenic conditions were necessary when the 0.32 mm ID column was installed because the low GC flow rate required long desorb times. The sub-ambient temperatures kept the sample in a tight slug which was essential for proper chromatographic peak shape.

The SGE metal jet was well suited for 0.53 mm ID columns and the HP 5971A. It eliminated the need for make-up gas (necessary with the glass jet). MS Engine, the differentially pumped mass spectrometer was able to accommodate high flow rates (10 ml/min) without a jet separator.

The capillary direct interface with cryofocussing allowed for low level calibrations at concentrations five times lower (0.04 ppb instead of 0.2 ppb) than the 0.53 mm ID column with the metal jet. The initial cost of the capillary direct configuration was less than the jet configuration. However, maintenance costs of the LN\textsubscript{2} cooled capillary configuration were much higher. The jet configuration was easier to install and better tolerated water carry-over from the purge and trap.

By demonstrating that all three configurations are amenable to volatile analysis, the choice of set-up will be dependent on the sensitivity required. The 0.53 mm ID columns will have much higher capacity (>200 ng on column). The 0.32 mm ID columns are more useful at lower concentrations (<200 ng). Thus, the 0.32 mm ID columns are most useful for high sensitivity analysis, where the 0.53 mm ID columns can be used for the analysis of drinking water and waste water.
Figure 1. Chromatograms for 1 ppb (25 ng) purged standards.
"DETERMINATION OF OFF-ODORS AND OTHER VOLATILE ORGANICS IN FOOD PACKAGING FILMS BY DIRECT THERMAL ANALYSIS-GC-MS" Thomas G. Hartman, Joseph Lech and Robert T. Rosen; Center for Advanced Food Technology, Cook College, Rutgers University, New Brunswick, New Jersey 08903

This investigation was conducted to determine the compounds responsible for an off-odor in a polypropylene food packaging film manufactured from a recycled resin feedstock. The off-odor was not present in films made under identical conditions using virgin resin. Therefore, it was our intention to obtain a profile of the volatile organic compounds from both film samples and look for differences which may be the cause of the off-odor complaint. A Scientific Instrument Services (SIS) model TD-1 Short Path Thermal Desorber accessory combined with GC-MS was used for the direct thermal analysis of the food grade polypropylene packaging films. Films were placed directly in the desorption apparatus and heated (100°C, 10 min.) to outgas volatile and semi-volatile compounds from the polymer films. The desorbed compounds were then separated and identified by GC-MS. Compounds causing an off-odor in the recycled polypropylene film were easily identified by comparing chromatograms from good and bad films. Both films were found to contain over 175 volatile organic compounds. However, the film made from recycled resin was found to contain a cluster of approximately 25 peaks in the low boiling region of its gas chromatogram which were absent in the film made from virgin resin (Fig. 1,2). These peaks were identified as a mixture of C-7 to C-9 aliphatic and olefinic hydrocarbons with branched, linear and cyclic species present. Perhaps even more significant from a sensory viewpoint was the presence of N,N-dimethylformamide. The aromatic compound toluene was also detected. Thus based on this data it was judged that these compounds were the cause of the off-odor in the recycled films. Additional compounds which were found in both films included a range of phthalate ester plasticizers (Fig. 3), phenolic antioxidants such as butylatedhydroxytoluene (BHT) and 2,6-di-t-butylphenol (Fig. 4), paraffinic hydrocarbons used as mold release agents (Fig. 5) and a series of sesquiterpenoid compounds such as α-copaenes and α & β-ylangenes which may be fragrance compounds or UV-stabilizers.

Plastic films made for food packaging applications are primarily composed of high molecular weight polymers which are hence nonvolatile at temperatures below those which induce pyrolysis reactions. However, a multitude of low molecular weight volatile compounds are added to films as manufacturing aids and to impart functional properties into the products. Plasticizers and elastomers are used to promote flexibility and inhibit brittleness. Common plasticizers and elastomers include phthalates, adipates, other esters of dicarboxylic acids and fatty acid amide derivatives. Antioxidants are added to prevent oxidation of the film itself as well as to protect the foods stored within the films. Hindered phenols such as BHT, BHA, TBHQ and di-t-butylphenols are commonly employed for this purpose. Ultraviolet (UV) stabilizers are added to prevent "yellowing" of the films upon exposure to light. Compounds such as diphenylketones and various methoxylated derivatives are commonly used for this purpose. During the manufacture of these films various processing aids are employed. These include accelerators and cross-linking agents to promote polymerization and polymer strength and mold release agents such as paraffinic hydrocarbons or organosilicones which are used to prevent sticking to the rollers as the films are extruded. Still other additives may be added to prevent static build-up and fragrance compounds may be used to mask resulting off-odors in the films. Indeed, in some heavily plasticized films the total volatile organic fraction can be as high as 40 % w/w. Given the plethora of volatile organic compounds which are present in food packaging films it is not surprising that they readily migrate into foods stored therein often causing off-odors. In addition, there is presently much concern for the potential health risks associated with consuming food products with high levels of packaging borne migrants. The problems have recently become exacerbated by the increased use of packaging films as food wraps during microwave cooking. For these reasons it is obvious why there is a need for analytical methodology to study the volatile organic composition of packaging films. These investigations serve to define the additive composition of particular films and to assess the potential for migration of these species into foods. In addition this methodology is useful for general QA/QC testing, packaging compatibility studies or for comparing films from different vendors.
We acknowledge the Center for Advanced Food Technology (CAFT) Mass Spectrometry facility for providing analytical support. CAFT is an initiative of the New Jersey Commission on Science and Technology. This is NJAES publication number S-10569-1-91.

Fig. 1 Total ion chromatogram from polypropylene film with no off-odor from virgin resin

Fig. 2 Total ion chromatogram from polypropylene film with off-odor made from recycled resin

Fig. 3 Mass chromatogram for m/z 149 which is the base peak characteristic of phthalate ester plasticizers

Fig. 4 Mass chromatograms for the ions characteristic of the antioxidants BHT (m/z 220,205) and di-t-butyl phenol (m/z 206,191)

Fig. 5 Mass chromatogram of fragment ions characteristic of paraffinic hydrocarbons used as mold release agents
LOW PICOGRAM DETECTION IN THE FULL SCAN MODE
WITH THE HP 5970 MSD MASS SPECTROMETER

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The Hewlett Packard 5970 mass selective detector (MSD) demonstrates ample sensitivity for environmental waste analysis, but the lower limits of detection required for drinking water warrants improved sensitivity. Low picogram quantities can now be detected in the full scan mode by a modification to the detector. The quality control requirements of organic gas chromatography/mass spectrometry environmental analysis as found in the Federal Register¹ and also the U.S. EPA contract Lab program² can still be met. There is a 30 fold increase or more in absolute signal, which brings the MSD down into the picogram range of detection for full scan data acquisition, and much lower for selected ion monitoring. The improved sensitivity comes from a high energy dynode (HED), which has been described elsewhere³,⁴ to increase high mass signals that arise mostly from fast atom bombardment or liquid chromatography/mass spectrometry techniques. A conventional MSD detects positive ions by the production of secondary electrons released when incident ions impact the electron multiplier surface, while the HED uses post-acceleration of the positive incident ions to increase the sensitivity. The background subtracted mass spectrum of a 100 picogram injection of indeno(1,2,3-cd)pyrene made with an HED voltage of 5,000 volts, an electron multiplier voltage of 1800 volts, and a one microliter injection volume is shown in Figure 1. The MSD scanned at one cycle per second from 45 to 475 amu. A commercial environmental lab has placed the HED in an MSD for the analysis of volatile organic samples, and the quality control requirements in references 1 and 2 were satisfied, such as the twelve hour shift criteria and compound recoveries. The main benefit of the HED is increased sensitivity and lowered detection limits.

**Figure 1.** The background subtracted mass spectrum of 100 pg of indeno(1,2,3-cd)pyrene.

**References**

¹ Federal Register 49, 153, October 26, 1984.
Investigation into losses of dimethylphthalate from matrix and blank spikes in EPA Method 625 (extraction, GC/MS) analyses of wastewater in our laboratory pointed to base-catalyzed hydrolysis as the mechanism of loss. There is, in fact, a trend toward increasing $K_\text{OH}$ values for phthalate esters with decreasing alkyl group size and branching (1). EPA Method Study 30 (summarizing the results of a round-robin test of Method 625) suggests that hydrolysis of the lower molecular weight phthalate esters may occur during the base extraction, resulting in low recoveries. The report suggests that extraction be done as quickly as possible to minimize loss.

In an investigation of ways to improve the recovery for the phthalates in general, and DMPh in particular, we undertook several series of experiments. The first established the base pH-adjustment step as the point of loss. The others examined the effects of spiking concentration, pH, and delay time between basification and extraction on the observed recoveries of phthalate esters.

The purpose of the Series 1 experiments was to locate the point of loss for dimethylphthalate (DMPh) in Method 625 analyses. Experiments were set up to isolate the various parts of the extraction and extract concentration techniques used. In these experiments, DMPh was not detected (ND) at the method detection limit (MDL) of 4 µg/L in all the extracts for which the extracted water had been made basic. Recovery was greater than 95% for all the other samples. This strengthened the hypothesis that the cause of DMPh loss was base-catalyzed hydrolysis.

The purpose of the Series 2 experiments was to compare the effect of pH with that of spiking concentration. In these experiments, it was found that keeping the pH between 11.0 and 11.5 gave 30% recovery for DMPh, while adjusting to pH > 12 reduced its recovery to below the MDL. Detection at this MDL (4 µg/L) would be equivalent to 20% recovery. Samples for which the pH was between 11 and 11.5 were spiked at two concentrations, but recoveries for both were about 30%, suggesting that pH has a greater effect on DMPh loss than spiking concentration. Reproducibility between split duplicates was good.

The purpose of the Series 3 experiments was to compare the effects of pH on the recoveries of the six phthalates which are Method 625 analytes. The results are given in Figure 1. In these experiments, it was found that, while the greatest effect of pH is observed with DMPh, there is also a substantial effect on the recovery of butylbenzylphthalate (BuBzPh). Lesser effects were observed with diethylphthalate (DEPh) and dibutylphthalate (DBPh), while no effect was observed on the recoveries of dioctylphthalate (DOPh) and bis(2-ethylhexyl)phthalate (B2EHPh). The most dramatic change occurred between pH 11 and 11.5.

The purpose of the Series 4 experiments was to compare the effects of a time delay between pH adjustment and extraction on the recoveries of the same six phthalates. The results are given in Figure 2. In these experiments, it was found that reduction in recovery for DMPh and BuBzPh began within minutes of basification. Again, lesser effects were observed with DEPh and DBPh, and no effect was observed with DOPh and B2EHPh.
Examination of the results from these experiments suggests that for the best recoveries of phthalate esters from basic water extractions, the pH should be adjusted to just past pH 11 and the extractions should be performed within 15 minutes after the pH adjustment.

Notes

Figure 1. Extraction recoveries of Priority Pollutant phthalates from lab water versus pH.

Figure 2. Extraction recoveries of Priority Pollutant phthalates from basic(pH=11.5) lab water versus time delay between pH adjustment and extraction.
Direct Thermal Analysis of Plastic Food Wraps Using the Short Path Thermal Desorption System

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The purpose of this study is to determine the low level volatiles and semi-volatiles present on the surfaces of commercially available "microwave safe" plastic food wraps which have the potential to leach into food products during the microwave cooking process. The new "Short Path Thermal Desorption System" was utilized to permit the direct volatilization (sparging) of samples into the GC injection port without any prior solvent extraction or without the use of vapor traps. This technique permits the maximum sensitivity of analysis due to this direct injection technique with subsequent GC and GC/MS analysis.

The Scientific Instrument Services "Short Path Thermal Desorption System" was attached to the injection port of a H.P. 5971 GC/MSD. A J&W DB-5, .25mm x 25 meters, 0.25u film thickness capillary column was used for this study. The GC was used in the splitless mode at a flow rate of 0.5 ml/min.

Desorption Tube for Direct Thermal Analysis

Experimental

Three square inch samples of each of the four plastic food wraps was placed directly inside the 1/4" x 4" long GLT desorption tube. These tubes were then fitted to the Short Path Thermal Desorption System and fitted with a syringe needle. The sample was flushed with helium carrier gas and then autoinjected into the GC injection port. The heating blocks were heated to the sparging temperature indicated and closed around the desorption tube to ballistically heat the sample to the set temperature. This combination of heat and flow sparge any volatiles or semi-volatiles into the GC injection port were they are subsequently trapped in a narrow band at the front of the GC column which has been cryo-cooled to -40° C. The sample is collected for 10 minutes after which the GC is programmed at 10° per minute up to 280° C, to elute the products which are subsequently analyzed via the Mass Spectrometer.
Results and Discussion

Each of the four plastic food wraps produced its own distinctive pattern of peaks consisting of more than 100 peaks which vary in intensity from strong to very weak. Each of the chromatograms exhibit the total ion chromatogram for each of the four plastic food wraps.

Most of the peaks present exhibit 43, 57, and 71 masses which are indicative of the linear and branched paraffinic hydrocarbons which are commonly used as mold release agents during the manufacture of the plastic food wraps. These hydrocarbons and some of their corresponding alcohols comprise the bulk of the peaks detected.

In addition two major phthalates at 34.4 and 38.6 minutes were identified as diethyl phthalate and dibutyl phthalate in the Glad Wrap sample and several of the other plastic wraps.

Additional peaks present in the plastic food wrap samples were identified as BHT (Butylated Hydroxytoluene), a few cyclic hydrocarbons, and aromatics.

Conclusion

Utilizing the "Short Path Thermal Desorption System" it is possible to analyze plastic film samples, as well as other packaging materials, by sparging directly into the GC injection port, the volatiles eluted from the surfaces of these materials at elevated temperatures. This technique is unique in that it can be utilized to analyze the trace surface components and impurities present on these polymers which are not pyrolyzed or thermally affected at the relatively low temperatures used in this study. The amount of sample required for analysis is very small, and due to the direct injection and volatilization into the GC injection port, maximum sensitivity is attained via this technique. In addition labor time for sample prep is minimized and the use of solvents for extraction is eliminated with this technique.
Purge & Trap GC/MS Analyses of Volatile Organics in Imported Bottled Mineral and Spring Water

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The consumption of bottled water in the U.S. from both domestic and imported sources has been steadily increasing. The most recent incident which received much publicity was the finding of benzene in Perrier Water.

In order to obtain information with regard to current Good Manufacturing Practices, current/proposed Quality Standards and food labeling requirements, the FDA surveyed domestic and imported bottled water, including mineral and spring water for various contaminants including Volatile Organics (VOC's).

FDA is proposing to adopt as quality standards for bottled water (1,2,3) the Maximum Contamination Level (MCL) that EPA has established for seven of the following VOC's in public drinking water (4,5):

- Benzene 5 ppb
- Vinyl Chloride 2 ppb
- Trichloroethylene 5 ppb
- Trichloroethane 200 ppb
- Dichloroethylene 7 ppb
- 1,2-dichloroethane 5 ppb
- Carbon Tetrachloride 5 ppb

The limit for total trihalomethanes is already established by FDA at 100 ppb (6).

EPA method 524.1 (7), a Purge & Trap GC/MS procedure employing a 6 ft. packed GC column was used for the analyses of the water samples.

Samples (25ml) were spiked at 4 ppb with an internal standard and at 5 ppb with surrogate recovery compounds.

AutoQuan* procedure TCA was used to quantitate the mass spec runs. Average response values obtained from a four point calibration curve (1,4,10, & 20, ppb) were written into the library and used for quantitation and for comparison to the 4 ppb daily standard to determine instrument acceptability.

Thirty nine (39) samples of imported bottled mineral and spring water from thirteen (13) countries, representing thirty two (32) different brands were analysed for VOC's.

Twenty three (23) samples contained no reportable levels of VOC's and sixteen (16) samples contained reportable levels of seven (7) different VOC's. All reportable VOC's were well below the proposed limits:

- Chloroform (5) T-11 ppb
- Bromodichloromethane (1) 2 ppb
- 1,1,1-Trichloroethane (2) T
- Tetrachloroethylene (1) T

Only analytes not common to the blanks were reported.

The limit of quantitation was 1 ppb.
Values below 0.5 ppb are reported as None Detected.
Values between 0.5-1 ppb are reported as a Trace (T)
The 6 ft. 1% SP-1000 Carbopack B GC column provided good separation for all compounds of interest. Toluene (#28) was separated from Toluene-d<sub>s</sub> (#27 sur), 1,2-dichloroethane (#12) was separated from 1,2-dichloroethane-d<sub>s</sub> (#11 sur).

In peaks #19, 20, 21, 22, Benzene and dibromochloromethane were separated while 1,1,2-trichloroethane and dichloropropane were not completely separated, however, this did not present a problem in the analyses.

The average recovery for Toluene-d<sub>s</sub> (sur) over a three month period was 101% with a range of 85 to 116%. The average recovery for 1,2-dichloroethane-d<sub>s</sub> (sur) was 95% with a range of 81 to 114%.

REFERENCES

(5) Fed. Reg., 40 CFR Part 141, 142, 143, p 3528, 1/30/91
(6) 21 CFR 103.35 Subpart B
(7) EPA Method 524.1, Rev 3.0 1989
Eichelberger, J.W., & Budde, W.L.
Exploration of Ion-Molecule Reactions for Distinguishing Isomers of Polychlorodibenzo-p-dioxins

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Polychlorodibenzo-p-dioxins (PCDDs) have been known for their environmental hazard for decades. With the exception of the octachloro congeners, all the very toxic PCDD congeners have chlorine substitution at the 2, 3, 7 and 8 positions. In particular, much attention is focused on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) because of its extreme toxicity. For many years, GC/MS has been a standard method for analyzing TCDDs in environmental samples. However, isomers of TCDD often give identical or very similar mass spectra when traditional mass spectrometric techniques are used. By inducing collisionally activated dissociation (CAD) on [M]+ and [M]- ions of TCDDs, tandem mass spectrometry (MS/MS) has been shown to reduce inferences in complex environmental samples (1,2). The CAD technique did not offer isomer-specific identification for 2,3,7,8-TCDD because the fragmentation patterns of most TCDD isomers are very similar. In order to improve the specificity for analyzing PCDD isomers, we have explored the options of employing ion-molecule reactions occurring in the second quadrupole of a triple quadrupole mass spectrometer to distinguish the more toxic PCDD isomers from the less toxic ones.

All the reactant ions are produced in the ion source using ammonia chemical ionization (CI). Under the CI conditions, all the congeners with four chlorines or less give the [M-1]- ion as the predominant product with the exception of 1,2,3,4-TCDD which gives mostly the [M]- ion. The lower acidity of 1,2,3,4-TCDD is primarily due to the lack of the electron-withdrawing effect of having a Cl adjacent to H. Increasing the number of chlorines from four to five also decreases the acidity of PCDDs. No [M-1]- ion is found for the PCDD congeners with five chlorines or more. The electron capture product, [M]- ion, is the main product for the PCDD congeners.

The selection of the [M-1]- ion as the reactant for ion-molecule reactions can reduce interferences from compounds with relatively low acidity. We have studied hydrogen/deuterium (H/D) exchange reactions between DsO and [M-1]- ions of PCDDs with 4 chlorines or less. With the exception of 1,3,6,8-TCDD, exchange products are observed from all the isomers selected for this study. In the case of TCDD isomers, 2,3,7,8-TCDD can be distinguished from 1,2,3,4-, 1,3,6,8-, 1,2,7,8-, and 1,3,7,8-TCDD by its formation of only one exchange product (Figure 1). The exchange reactions are not observed for the [M]- ions of the higher chlorinated PCDDs. We believe that the lone-pair electrons on the [M-1]- ion are important to initiate an H/D exchange reaction.

Alcohol molecules are also useful for identifying 2,3,7,8-TCDD from the other isomers. When the alcohol neutral molecules react with the [M]+ ions of PCDDs, ion-molecule adduct complexes are formed. The reactivity increases from methanol to isopropanol; but it decreases dramatically to a very low level when isobutanol is used as a reagent. For the four alcohols used, the reactivity of 2,3,7,8-TCDD is consistently the lowest among the five selected TCDD isomers. The [M]+ ion of 2,3-dichlorodibenzo-p-dioxin (DCDD) also exhibits lower reactivity compared to the other isomers of DCDDs. Since the most probable charge site for the anion of 2,3-DCDD is the same as the one on 2,3,7,8-TCDD, we conclude that a charge site located adjacent to an oxygen atom of a PCDD anion is an unfavorable site for the formation of an adduct complex. The unique chemical characteristics of the [M]+ ion of 2,3,7,8-TCDD have been well demonstrated by our studies. These results encourage the continued search for better isomer-specific ion-molecule reactions.

References:


Acknowledgement:
This work is supported by the MSU Research Excellence Fund in Biotechnology.

Figure 1. Product spectra of H/D exchange reactions between D₂O and [M-1]⁻ of TCDD isomers.
COMPARISON OF POINT AND NON-POINT SOURCES OF PCDD AND PCDF TO SEDIMENTS AND FISH FROM THE HOUSATONIC RIVER AS DETERMINED BY GC/HRMS.

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Polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are highly toxic classes of compounds produced during combustion and as a by-product of various manufacturing processes. The combustion sources result in atmospheric transport and deposition creating a non-point source to the environment, while the by-products are often discharged with wastewater creating point sources.

The Housatonic River flows through a series of impoundments in Southwestern Massachusetts and Western Connecticut into Long Island Sound. Much of the river is contaminated with polychlorinated biphenyls (PCB) at concentrations up to 60 ppm. The PCB were from a point source and PCDF are known contaminants in PCB. There are also several paper mills which discharge into the river. Thus sediments and fish from this river offer a unique opportunity to compare the environmental importance of PCDD and PCDF from these point sources with atmospheric input from the industrial Northeast.

Sediment and fish samples were taken from the impoundments of the river (from upstream to downstream respectively) at Center Pond (C), Woods Pond (W), Rising Pond (R), Falls Village Dam (F), Bulls Bridge Dam (B), Upper Lake Lillinonah (U), Lower Lake Lillinonah (L), and Lake Zoar (Z). Comparison samples were taken from the Shepaug River (R) (a Housatonic tributary) and West Side Pond (P) (a small isolated lake in the same region).

Samples were analyzed via a semi-automated modification of EPA method 8290 utilizing gas chromatography/high resolution mass spectrometry in the electron impact mode. Ions were selected for monitoring at mass resolution of 10,000 to minimize contribution to the ion channel for PCB remaining in the sample after clean-up.

Concentrations of the dioxins and furans ranged from 24 ppb PCDD and 44 ppb PCDF in Woods Pond to 110 ppt PCDD and 44 ppt PCDF in Center Pond. The concentrations of PCDD/F normalized to the organic carbon in the sediment is shown in Figure 1. Note the large jump for Woods Pond (just beyond the point PCB source) and a smaller jump for Lake Lillinonah (after a non-point input from the industrial area around Danbury).

Figure 2 is a principal components plot of the congener profile normalized to the total PCDD/F concentration for that sample. The first two factors are plotted. Note how the profile shifts for the Woods Pond samples and then gradually returns to "normal" as sample sites move down stream. These results show that the effect of a point source can be seen in sediments taken from an industrial area with a non-point or atmospheric profile.
Figure 1. Average total concentrations normalized to organic carbon for each site.

Figure 2. Plot of the first two principal components of the congener profiles normalized to the total PCDD/F concentration.
THE DETERMINATION OF COPLANAR PCBs, PCDDs, AND PCDFs IN MOTHER'S MILK BY HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY

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INTRODUCTION

Polychlorinated biphenyls (PCBs) have been identified in virtually every part of the global ecosystem, including human and animal tissues, milk, sediment, and numerous other matrices. Because of the chemical and physical stability of PCBs, they tend to bioaccumulate in nature. Toxicological investigations of PCBs are complicated by the potential for exposure to up to 209 isomers. However, selected coplanar PCBs, particularly 3,3',4,4'-tetrachlorobiphenyl (IUPAC #77), 3,3',4,4',5-pentachlorobiphenyl (IUPAC #126), and 3,3',4,4',5,5'-hexachlorobiphenyl (IUPAC #169) have been found to elicit toxic responses similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). As a result, there is an interest in developing information on human body burdens of these compounds in addition to that of the 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs).

The coplanar PCB isomers are typically observed in human tissue at much lower concentrations than other PCB isomers. Therefore, accurate quantification of the coplanar PCBs is very difficult by standard GC/ECD methods. It is possible, however, to separate the coplanar isomers from the non-coplanar PCBs through the use of carbon adsorbents. This procedure isolates the coplanar PCBs, PCDFs, and PCDDs, in the same carbon column fraction so that they may be determined in a single HRGC/HRMS analysis.

EXPERIMENTAL

A quality control mother's milk pool was prepared from volunteers in the Kansas City area. Milk samples from five mothers were composited and homogenized with mechanical mixing. Approximately 50 grams of milk was fortified with nine $^{13}$C$_{12}$-labeled 2,3,7,8-substituted PCDD/Fs, and three $^{13}$C$_{12}$-labeled coplanar PCBs as internal standards for the determination of PCDD/Fs and coplanar PCBs, respectively. The fortified milk samples were then mixed with an aqueous solution of sodium oxalate, ethyl ether and ethanol, and extracted with hexane for 30 min. The hexane extract was recovered and concentrated to a constant weight for the determination of extractable lipid. The lipid residue was diluted in hexane and subjected to an acid silica gel slurry and neutral/acid silica gel chromatography column. The subsequent column chromatography cleanup steps included neutral alumina and Carbopack C/Celite. The extract was then concentrated, fortified with recovery internal standard, and concentrated further to a final volume of 5 µL.

The HRGC/HRMS analysis of the mother's milk extracts were conducted on a 60 meter DB-5 capillary column coupled directly to a VG70S high resolution mass spectrometer operated in the selected ion monitoring mode. Several modifications to existing methodologies were made to allow simultaneous detection and quantification of the coplanar PCBs along with the PCDDs and PCDFs. One modification was necessary to resolve hexachlorobiphenyl isomer 169 from 1,2,3,7,8-PeCDF. These two compounds coelute and require a mass resolution of approximately 50,000 to separate their isobars at m/z 358, 360, and 362. The M+4 peak of 1,2,3,7,8-PeCDD can be totally obscured in the case where the PCB
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isomer 169 is present in higher concentrations than the dioxin congener. In order to avoid overlap of the responses of these compounds it is necessary to choose alternate ions for PeCDD from those suggested in the standard methods. Monitoring m/z 354 and 356 (M+ and M+2 respectively) for PeCDD instead of m/z 356 and 358 (M+2 and M+4) eliminates all overlap of hexachlorobiphenyl isomer 169 with 1,2,3,7,8-PeCDD.

Another modification to existing methods involves monitoring 12 additional ions necessary to determine the PCBs and the corresponding 13C-PCB internal standards. The PCB isomer 77 masses are monitored in a separate group which is inserted into the normal experiment before the TCDD/TCDF ion group. The PCB isomer 126 masses are added to the TCDD/TCDF ion group. The addition of four extra masses into this group does not compromise the sensitivity TCDD and TCDF. PCB isomer 169 masses are monitored during the elution of the PeCDDs and PeCDFs. Again, the addition of these extra masses does not affect the overall sensitivity.

RESULTS

The procedure outlined above yields accurate and precise data for the determination of PCDDs, PCDFs, and coplanar PCBs in mother's milk at the low parts per trillion level. The internal standard recoveries ranged from 65 to 82% for 13C-labeled PCDD/Fs and from 64 to 72% for 23PCBs. Accuracy ranged from 93 to 111% for PCDD/Fs and 73 to 96% for PCBs in fortified milk samples. Replicate analysis of control milk samples yielded percent standard deviations of 5 to 21% for PCDD/Fs and 10 to 31% for coplanar PCBs. These data are well within the performance criteria specified in most PCDD/F methods.

<table>
<thead>
<tr>
<th>Method Accuracy for Compounds Spiked into Control Milk (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SPK LEVEL</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td><strong>COMPOUND</strong></td>
</tr>
<tr>
<td>2378 TCDF</td>
</tr>
<tr>
<td>2378 TCD</td>
</tr>
<tr>
<td>2378 PECDF</td>
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<tr>
<td>234678 9OCDF</td>
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<tr>
<td>234678 9OCDF</td>
</tr>
<tr>
<td>Tetra PCB #77</td>
</tr>
<tr>
<td>Pentra PCB #126</td>
</tr>
<tr>
<td>Hectra PCB #189</td>
</tr>
</tbody>
</table>

a. Total pg spiked into 40 ml control milk. Typical amount of lipid was 1.3 g.
b. Percent accuracy = ([pg/g found in method spiked - pg/g found in unspiked control]/pg/g spiked) X 100 %
c. 123478 and 123678 HXCDD reported as the sum of both isomers.
DIFFERENTIATION OF AROCLORS IN ENVIRONMENTAL SAMPLES USING NEGATIVE ION CHEMICAL IONIZATION (NICI) MASS SPECTROMETRY

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Introduction

Environmental samples suspected of containing polychlorinated biphenyls (PCB) and analyzed by EPA Method 8080 frequently contain non-PCB components, such as phthalates, PAH's, or organochlorine pesticides. The presence of these interferences can often obscure the GC/ECD patterns and cause problems in differentiating the Aroclor types by visual inspection. Since Method 8080 requires the identification of Aroclor types in order to trace the sources of PCB occurrences, NICI detection was used to provide additional parameters for discriminating PCB congeners from interferences. In this study, a pattern recognition method has been developed to classify the types of Aroclors for environmental samples. A computer program written in BASIC has been implemented to facilitate Aroclor classification using the NICI ion abundance measurement for PCB congeners.

Experimental

NICI spectra for PCB samples in this study were obtained with a Hewlett-Packard 5985B GC/MS system equipped for EI and CI operation. Samples were introduced via a 30 m x 0.25 mm DB-5 fused silica column with helium as carrier gas. The column temperature was programmed from 130°C to 180°C at 5°C/min, then increased to 250°C at 2°C/min. The flow rate of reagent gas (methane), introduced through a heated transfer line, was regulated to maintain a source pressure of 0.4 torr. The ion source temperature was 100°C. The electron energy was set at 200 volts and the emission current at 300 uA. Nine Aroclor standards, including 1016, 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268, were analyzed for a concentration range of 0.2 to 100 ppm. Using selective ion display, the ion abundances of the prominent peak in the chlorine cluster were measured for eight homologs \((\text{Cl}_2-\text{Cl}_6)\) within the PCB retention window for each
standard. These NICI measurements on Aroclor standards were used as training data set to develop classification methods for environmental samples. PCB contaminated oil or soil samples were either extracted or diluted with hexane and analyzed in the same manner as the standards.

Results and Discussion

The relative ion abundances (RIAs), defined as the abundance of each homolog with respect to the sum of all homologs, were calculated for eight selective ions (m/z 221, 256, 292, 326, 360, 394, 430, and 464). The distances between Aroclor classes are measured in a 7-dimensional simplex space defined by the RIAs. We used the Mahalanobis distance function to measure separation between centroids of the Aroclor classes. Aroclor classification uses the smallest number of RIAs. The number of RIAs was selected by (1) ordering from largest to smallest the distance ratios of "between class" to "within class" for each RIA, and (2) the accumulative minimum distance among all the classes.

We developed linear discriminant functions (LDFs) to classify samples into the nine Aroclors based on 74 samples in the Aroclor training set. The first step of a sequential classification method calculated LDFs using six RIAs for ions m/z 430, 464, 394, 326, 256, and 360 to identify Aroclors 1016, 1268, and remaining Aroclors. Additional classification was necessary for samples classified as remaining Aroclors. Updated LDFs identified Aroclors 1221, 1232, 1242, 1248, 1254, 1260, and 1262. These updated LDFs used different set of RIAs (for ions m/z 430, 394, 326, 292, and 360) for classification variables and a different covariance matrix than those in the first step.

This sequential classification method classified all Aroclors in the training set correctly. A set of 15 environmental samples with known Aroclor types were also correctly classified.

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Analytical procedures and a quality assurance plan have been developed for the determination of coplanar polychlorinated biphenyls (PCBs) #77 (3,3',4,4'-tetrachloro-), #105 (2,3,3',4,4'-pentachloro-), #126 (3,3',4,4',5-pentachloro-) and #169 (3,3',4,4',5,5'-hexachloro-) in biological tissue at target level of quantification of 5 pg/g. The methodology is based upon isotope dilution HRGC/HRMS and is both analogous to and compatible with rigorous protocols developed for PCDD/PCDF determinations (1) as well as other environmental contaminants such as PCBs and pesticides (2).

The protocol for the isolation of coplanar PCBs was based upon chromatography on carbon (AMOCO PX-21) which has been suspended upon silica gel, however the exact procedure was determined by the analyte list. For instance if PCDD/PCDF were the only other analytes to be quantified, then soxhlet extraction and sample cleanup on a sulfuric acid/silica gel column was used, but if pesticides and PCBs were to be analyzed then soxhlet extraction and sample cleanup by gel permeation chromatography and micro-silica gel chromatography was used. In each case, extracts (0.5 mL in isoctane) were transferred to a 500 mm X 5 mm column containing 350 mg of carbon on silica gel (100 mg/g) with three 0.5 mL portions of hexane and eluted with an additional 18 mL hexane to remove non-coplanar PCB and pesticides. The column was then washed with 22.5 mL methylene chloride/benzene (1:1, v:v) to obtain coplanar PCBs. Finally, the column was eluted with 25 mL toluene in a reverse flow direction to obtain PCDD/PCDFs.

Analysis is accomplished using HRGC (30m X0.25 mm DB-5) and HRMS (R=5000) operating in the MID mode. Quantification of PCBs #77, #126, and #169 are relative to 13C12 analog internal standards. Quantification of PCB #105 is relative to PCB 13C12 #126. Rigorous QA/QC criteria which include GC elution time, signal to noise ratios, and ion abundance ratios have been established to assure...
high quality data (3). QA/QC criteria for the performance of the instrument follows that established for PCDD/PCDF determinations.

An analysis of composites of fish from the Great Lakes and Atlantic bottlenose dolphins from the U.S. east coast has been completed. The concentration of analytes in Lake Ontario lake trout, for instance, were; 22.5, 486, 6.96, and 0.62 ng/g lipid for PCB # 77 #105, #126, and #169 respectively. The mean concentrations for five dolphins were lower; 0.68, 251, 0.60, 0.27 pg/g lipid for the same PCBs. The observation of low coplanar PCB concentrations is consistent with data that show the concentration of other coplanar analytes such as PCDD and PCDF are also very low when compared to fish. Relative to total PCB concentrations, coplanar PCB in dolphin samples comprise a much smaller proportion than in fish. The reason for the differences is yet unknown, but may be due to differences and physical/chemical properties of individual PCBs, which results in different PCB compositions to which the animals are exposed, and/or to differences in metabolic capabilities. The capacity for biotransformation of PCBs has been found to increase in the order fish, open water cetaceans, harbor porpoises and seals.

References


IDENTIFICATION OF A BASE BREAKDOWN PRODUCT OF A NEW HERBICIDE
BY HIGH RESOLUTION MASS SPECTROMETRY AND PROTON NMR.

By
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A new herbicide F 6285 was placed in a 0.5 N solution of NaOH. The resulting product was extracted and submitted for NMR and MS analysis.

Low resolution (RP • 1,000) El mass spectral data determined the mass of the product to be m/z 320 (FIGURE 1). The isotope distribution indicated that 2 Cl's and 1 S were present. The mass of the parent herbicide was known to be 386 u, therefore some base breakdown had occurred and structural identification was necessary to determine a mechanism for this new product.

High resolution (RP = 10,000) El peak matching data determined the elemental composition to be C10 H10 N4O2SCl2. The results are below:

Measured Mass 319.99011
Theoretical Mass 319.99957
Difference (u) 0.00044
Difference (ppm) 1.4

The Proton NMR results indicated that two labile protons could be present in the base breakdown product. See FIGURE 2 (bottom).

Proton NMR spectra of F6285 base breakdown product before (bottom) and after the addition of deuterated pyridine (top).
To ascertain the number of labile protons an aliquot of CD₃OD (0.25 ml) was added to the sample and a low resolution EI mass spectrum was obtained (FIGURE 3). The results indicated that only one labile proton was indeed present in our base breakdown product.

To cross-check our conclusion that there is only one labile proton present in our base breakdown product, an aliquot of deuterated pyridine was added to the CDC₁₃ solution and a second proton NMR spectrum was obtained. We had suspected that some of the peak broadening (8.5 and 6.9 ppm) in the original NMR data was due to a small amount of HCl present in our CDC₁₃ solvent. Because of our relatively small amount of material (~2 mg) the peak broadening due to the HCl was evident.

The results are observed in FIGURE 2 (top) The peak at 8.5 ppm was sharpened and the peak at 6.9 ppm disappeared. The peak at 6.9 ppm was concluded to be the labile proton, and the structure and subsequent mechanism for the base breakdown product is shown in FIGURE 4.

**Proposed mechanism for the base breakdown product of F6285.**
LC/MS AS A COST EFFECTIVE METHOD FOR MULTIRESIDUE ANALYSIS OF HERBICIDES AND METABOLITES IN CROPS, SOIL, AND WATER

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INTRODUCTION

The demands for analytical methods to detect residues of crop protection chemicals in our environment stressed the need for multiresidue techniques. Thermospray LC/MS offers both the liquid chromatographic separation needed for thermally labile pesticides, high sensitivity, and mass selectivity required for trace level multiresidue analysis. In our recent applications, we used the technique as a stand-alone, primary method for multiresidue analysis of thermally labile sulfonylurea herbicides and metabolites in crops, soil, and water (1, 2). Using the mass spectrometer in the selected ion mode offers a universal selective detection which minimizes the need for sample clean-up and simplifies method development. Simple sample preparation will increase sample throughput which saves time and minimizes solvent waste. In addition, it will improve recovery by reducing analyte losses during the clean-up steps. Using the mass spectrometer for detection offers a build-in structure confirmation of the target compounds which eliminate the chance for false positive. This report describes the application of LC/MS for residue analysis of three sulfonylurea herbicides, sulfometuron methyl, the active ingredient of the noncrop land herbicide OUST and chlorsulfuron, the active ingredient of the cereal herbicide GLEAN and bensulfuron methyl, the active ingredient of the rice herbicide LONDAX in soil down to 50 ppb. In addition, the report includes residue methods for three sulfonylureas in wheat grain at 50 ppb level and another method for the analysis of sulfometuron methyl and metabolite in water at lower levels.

EQUIPMENT

The HPLC system consisted of a VARIAN model 5560 liquid chromatograph equipped with a constant-flow pump, a variable wavelength detector (VARIAN, Instrument Group, Walnut Creek, CA), and an Alltech Spherisorb ODS column, 4.6 mm i.d. x 25 cm (Alltech/Applied Science, IL). The mass spectrometer was a Finnigan model TSQ70 triple stage quadrupole instrument with the ICIS data system. The LC/MS interface was a Finnigan TSP2 thermospray with discharge electrode and filament ionization (Finnigan MAT, San Jose, CA). The liquid chromatographic conditions were optimized to separate the herbicide using gradient elution system of acetonitrile in 0.1M acetic acid. A simple extraction procedure was used to extract the herbicides from crops and soil without further clean-up. A typical sample preparation includes extracting a 10 gm sample three times with 10 mL aliquots of 80% acetonitrile in water followed by filtration.

Extract aliquots were concentrated 5/1 prior to the LC/MS analysis. The thermospray LC/MS interface temperature and mass ions were selected to maximize sensitivity and selectivity. The ammonium acetate solution required for thermospray ionization was added post column to maintain the chromatographic separation of sulfonylureas.

RESULTS AND DISCUSSION

An LC/MS reconstructed ion chromatogram for a standard mixture of sulfometuron methyl, chlorsulfuron and bensulfuron standards (0.1μg/mL) is presented in Figure 1. The
Figure indicates a stable ion signal and good chromatographic separation. A detection limit of 0.01 µg/mL is easily obtainable due to the good signal-to-noise ratio. Figure 2 represents the LC/MS total ion chromatogram of a 0.05 ppm fortified soil extract. The chromatogram shows no interference from the soil matrix at the retention time of the three sulfonylureas. The recovery of the three sulfonylureas in soil was determined by comparing the amount detected by LC/MS in the final extract of the fortified samples to the original fortification level. The recoveries were between 88-115% for the three compounds, sulfometuron methyl, chlorsulfuron, bensulfuron methyl at levels of 0.050 - 0.200 ppm in soil and were 75-95% in wheat grain at levels of 0.050 - 0.500 ppm.

LITERATURE CITED


A COMPARISON OF LOW RESOLUTION AND HIGH RESOLUTION MASS SPECTROMETRIC TECHNIQUES FOR THE DETERMINATION OF N-NITROSODIMETHYLAMINE IN ENVIRONMENTAL SAMPLES

Ontario Ministry of the Environment, Rexdale, Ontario, Canada M9W 5L1

The Ontario Ministry of the Environment analyzes N-nitrosodimethylamine (NDMA) in a variety of aqueous matrices including raw and treated drinking water, surface water, groundwater, sewage treatment plant influent and effluent, industrial effluent and impinger water. The analysis of NDMA in matrices that vary widely in composition requires a protocol that is selective as well as sensitive. The protocol must also be simple and rugged in order to maintain a high level of productivity.

The sample preparation procedure (1) consists of an extraction step (with dichloromethane) combined with acid/base partitioning to remove basic and acidic components respectively followed by concentration of the extract containing the remaining neutrals. Quantitation was performed by a standard isotope dilution technique using $d_6$-NDMA.

Analysis by GC/LRMS consisted of monitoring the quantitation ion at m/z 74 and the qualifying ion at m/z 42 on a VG Trio-2 using a 30m DB-1701 column. The ions monitored for the $d_6$-NDMA were m/z 46 and m/z 80.

Analysis by GC/HRMS consisted of single ion monitoring at 7,000 RP on a VG ZAB-2F using a 30m DB-1701 column. The ions monitored were m/z 74.0480 for NDMA, m/z 80.0857 for $d_6$-NDMA and m/z 68.9952 for PFTBA (lockmass).

Drinking water samples were analyzed by GC/LRMS and GC/HRMS (1). Although these samples are considered to be relatively clean, their chromatograms showed interfering peaks and the 42:74 ion ratio varied widely. The acceptable window for this ion ratio was set at +/- 20% of the value obtained from a calibration standard run on the same day. It was shown that the presence of chemical interferences made the 42:74 ion ratio criterion unreliable and that analysis by GC/HRMS was preferable.

Other aqueous matrices were analyzed using the same sample preparation protocol. These included sewage treatment plant (STP) influent and effluent, groundwater and industrial effluent. For samples containing high concentrations of NDMA, smaller aliquots were taken and diluted accordingly with HPLC-grade water so that the ratio of NDMA:$d_6$-NDMA would be close to 1:1. The sample extracts were analyzed by GC/LRMS and GC/HRMS. The results are shown in Table 1.

These data also show that the 42:74 ion ratio criterion is unreliable and that interferences are present at m/z 74 as well as at m/z 42. It is also possible to have interferences with the $d_6$-NDMA at m/z 80.
TABLE 1

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>GC/LRMS</th>
<th>GC/HRMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) spiked HPLC water</td>
<td>0.49(i) ug/L</td>
<td>0.42 ug/L</td>
</tr>
<tr>
<td>(B) STP influent</td>
<td>0.31(i)</td>
<td>0.27</td>
</tr>
<tr>
<td>(C) STP influent</td>
<td>0.20(i)</td>
<td>0.24</td>
</tr>
<tr>
<td>(D) STP influent</td>
<td>0.43</td>
<td>0.40</td>
</tr>
<tr>
<td>(E) STP influent</td>
<td>0.11(i)</td>
<td>0.020</td>
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<td>(F) STP influent</td>
<td>0.034(i)</td>
<td>0.043</td>
</tr>
<tr>
<td>(G) STP influent</td>
<td>0.058(i)</td>
<td>0.020</td>
</tr>
<tr>
<td>(H) groundwater</td>
<td>ND</td>
<td>0.037</td>
</tr>
<tr>
<td>(I) groundwater</td>
<td>ND</td>
<td>0.088</td>
</tr>
<tr>
<td>(J) groundwater</td>
<td>1600(i)</td>
<td>170</td>
</tr>
<tr>
<td>(K) groundwater</td>
<td>840(i)</td>
<td>170</td>
</tr>
<tr>
<td>(L) industrial effluent</td>
<td>0.37(i)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

i=interference (incorrect ion ratio)
ND=not detected (no peak)

In subsequent studies of these interferences, another groundwater sample was analyzed by full scan GC/MS. Chlorobenzene and ethylbenzene were found to coelute with the NDMA on a DB-1701 column. M/p-xylene and o-xylene elute 0.2 min and 0.8 min respectively after the NDMA. These interferences are compounds that have minor ions in common with NDMA but are present in much higher concentrations. Chlorobenzene and ethylbenzene have a minor ion at m/z 74 but no ion at m/z 42. Their presence will affect the 42:74 ion ratio as well as the quantitation. Interferences having ions at m/z 42 only will affect the 42:74 ion ratio but not the quantitation. On a DB-5 column, toluene coelutes with NDMA.

Another sample contained N,N-dimethylformamide (DMF) which elutes after NDMA. If present in sufficient quantities, the M+1 (m/z 74) of DMF will interfere with NDMA.

The chemical interferences that have been identified in these samples will adversely affect the analyses of NDMA if they are done by GC/LRMS with the present level of sample cleanup. Further cleanup steps would be necessary if analysis of the underivatized NDMA by GC/LRMS is to become a reliable alternative to GC/HRMS. However, this would also adversely affect productivity.

These results demonstrate the superior selectivity of HRMS over LRMS. With GC/HRMS on a ZAB-2F, a method detection limit of 5 ppt at 7,000 RP can be obtained on a 1 L water sample. The instrument detection limit is <1 ppt.

INTRODUCTION

The fungus, Fusarium moniliforme, can cause leukoencephalomalacia (LEM) in horses (1) and has been shown to be a rat carcinogen (2). Fumonisin B₁ is a member of a family of mycotoxins recently isolated from cultures of the fungus F. moniliforme. Gelderblom et al. (3) have shown that Fumonisin B₁ displays toxic effects when given to rats. Marasas et al. (4) induced LEM in horses by intravenous injections of Fumonisin B₁. These reports suggest that Fumonisin B₁ could be the toxic component of F. moniliforme. Currently, HPLC with fluorescence detection is one of the most promising techniques being developed for the routine determination of Fumonisin B₁ (5). Mass spectrometry (MS) is also being investigated for use in the characterization of Fumonisin B₁. Both Bezuidenhout et al. (6) and Plattner et al. (7) have described the identification of Fumonisin B₁ using various techniques including liquid SIMS.

We have investigated the utility of thermospray mass spectrometry (TSM), fast atom bombardment mass spectrometry (FABMS), and electrospray MS (ESMS) for the analysis of Fumonisin B₁. In addition, we have analyzed two different standards of Fumonisin B₁ as well as a corn culture extract which contained Fumonisin B₁. This report describes the results of our efforts, showing that ESMS as well as FABMS and FABMS/MS provide useful data for the characterization of Fumonisin B₁.

EXPERIMENTAL SAMPLES

Two standards of Fumonisin B₁ were obtained from two different sources: Sigma and South Africa. The Sigma (Sigma Chemical Co., St. Louis, MO) standard was dissolved in 50/50 acetonitrile/water to give a solution that was nominally 100 µg/ml. The South African standard was received as a solution from Misty Conder (Arkansas Livestock and Poultry Commission) and was approximately 5 µg/ml in 50/50 acetonitrile/water. In addition, a purified extract from a corn culture containing Fumonisin B₁ was obtained from Ron Plattner (USDA/ARS, Peoria, IL); the concentration of this extract was adjusted to approximately 500 µg/ml in 50/50 acetonitrile/water.

THERMOSPRAY

The TS mass spectra were obtained using a Finnigan MAT TSQ 70 triple quadrupole mass spectrometer equipped with a Finnigan TS interface and source. Analyses were performed in the positive ion, discharge-off and filament-off mode. Flow injection analyses were performed using an Isco model LC-5000 syringe pump connected to a Rheodyne 7125 injector equipped with a 20 µl sample loop. The mobile phase was 0.1 M ammonium acetate in water pumped at 1.25 ml/min. Typically, the vaporizer was set to 90 °C and the block was set to 220 °C. The mass spectrometer was scanned from m/z 200 to m/z 800 at a rate of 1 sec/scan.

ELECTROSPRAY

The electrospray mass spectra were obtained using a Delsi/Nermag R3010 triple quadrupole mass spectrometer equipped with a Delsi/Nermag electrospray interface and source. The samples were pumped into the source at a flow rate of 1 µl/min. The mass spectrometer was scanned from m/z 100 to m/z 1000 at a rate of 4 sec/scan.

FAB

The FAB mass spectra were obtained using a Finnigan MAT TSQ 70 triple quadrupole mass spectrometer equipped with a Finnigan FAB source and an Ion Tech fast atom gun. Xenon was utilized in the primary atom beam at energies of 8 to 10 KeV. Thioglycerol was used as the matrix. Typically, 1 µl of sample was applied to the FAB probe tip after the matrix was applied, and the mass spectrometer was scanned from m/z 200 to m/z 800 at a rate of 1 sec/scan.

FABMS/MS analyses were performed by using the same conditions, but with argon set to about 0.5 mtorr as the collision gas and a collision energy of either 30 or 50 eV. To obtain daughter ion spectra, MS1 was set to pass the selected parent ion while MS2 was operated under full scanning conditions.
RESULTS AND DISCUSSION

Under TSMS conditions, Fumonisin B₁ gave an [MH]⁺ ion at m/z 722, but the signal was very weak and the relative intensity of the [MH]⁺ ion was less than 10%. Multiple fragment ions were also observed by TSMS.

Using ESMS, each standard showed a strong [MH]⁺ ion at m/z 722 as the base peak in the mass spectrum. Figure 1 shows the ES mass spectrum of the Sigma standard; the peak at m/z 744 is the [MNa]⁺ ion, and the peak at m/z 760 is the [MK]⁺ ion. The ES mass spectrum for the South African standard was similar to the one shown in Figure 1, except that in addition to the ions described for Figure 1, the mass spectrum included peaks at m/z 766 and m/z 788, corresponding to the [M + 2Na - H]⁺ ion and the [M + 3Na - 2H]⁺ ion, respectively.

FABMS provided strong [MH]⁺ ions at m/z 722 for each of the two standards. Figure 2 shows the FAB mass spectrum for the Sigma standard. The FAB mass spectrum obtained from the analysis of the corn extract sample also showed a base peak at m/z 722 indicating that Fumonisin B₁ was a major component in the sample. In addition, a new peak at m/z 564 was observed; the m/z 564 peak was assumed to be due to some unknown impurity in this sample. FABMS/MS analysis of the standards and sample was also performed. CAD of the [MH]⁺ ions for Fumonisin B₁ yielded daughter ions attributed to side chain cleavage and water losses. Similar results were obtained from the CAD analyses of the two standards and the corn extract sample. CAD of the m/z 564 ion from the corn extract sample provided data that suggest that the impurity which yields the m/z 564 ion has a structure in which one of the side chain groups in the Fumonisin B₁ molecule is replaced by a H atom.

CONCLUSIONS

While TSMS was not found to be suitable for the analysis of submicrogram quantities of Fumonisin B₁, both FABMS and ESMS provided useful data for nanogram quantities of this compound. In addition, FABMS/MS was found to be useful for the confirmation of Fumonisin B₁ in a corn culture extract. FABMS/MS was also utilized for characterizing an impurity in the corn extract sample.

REFERENCES

A CONCISE METHOD FOR THE DETERMINATION OF PESTICIDES AND THEIR METABOLITES IN GROUNDWATER.

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The wide use of pesticides in agriculture, their persistence at trace levels in the environment and the concern over their health risk effects have prompted an interest in the development of multi-residue analytical methods. The objective of this study was to develop a single and concise method to survey groundwater contamination in selected agricultural areas of the state of Minnesota. The study includes atrazine, alachlor, fonofos, phorate, chlorpyrifos, terbufos, carbofuran and most of their known degradation products.

A study comparing solid phase extraction (SPE) to liquid-liquid extraction indicated neither of the methods was suitable for good recoveries of all compounds under investigation. Fig. 1. shows recoveries for the phosphate residues.

It has also been shown that atrazine is better recovered by solid phase extraction while its degradation products de-ethylatrazine and de-isopropylatrazine are obtained in higher recovery by liquid-liquid extraction. Therefore, triplicate samples of groundwater were collected. One sample was pumped through a C-18 SPE cartridge, eluted first with ethyl acetate followed by a second elution with acidified methanol to recover hydroxyatrazine. The ethyl acetate extracts were concentrated under nitrogen to 0.2 mL, spiked with internal standard solution and analyzed. The second sample was subjected to liquid-liquid extraction using dichloromethane. The extracts were solvent exchanged to ethyl acetate and concentrated to 0.2 mL, spiked with the same internal standard solution and analyzed. Carbofuran and its degradation products, and de-methoxymethylhydroxyalachlor were measured in the third sample by derivatizing another SPE extract with trifluoroacetic acid anhydride to yield stable compounds suitable for GCMS analysis.

Analyses were performed on a Hewlett-Packard (HP) 5890 A gas chromatograph equipped with a 30 m x 0.25 mm DB-5 (J+W Scientific) capillary column coupled to an HP 5988 mass spectrometer in El mode. Injections of 1 μL were made by an HP 7673A automatic sampler. Separation was accomplished by a multistep temperature program. Two ions from each compound were selectively monitored over the appropriate retention window. The most abundant ion for each analyte was used.
for quantitation and the second most abundant for confirmation as shown in Fig. 2. Quantitation was done by the internal standard method using the Aquarius subroutine of the Hewlett-Packard RTE-A data system. The relative response factors were linear from 0.1 to 10 ng injected. Confirmation of the presence of any analyte was based on the relative retention time, the presence of the confirmation ion and the ratio of the abundances of the confirmation to quantitation ions. The detection limit of these compounds range from 10 to 100 pg injected. Carbofuran and its degradation products were screened for their presence in SPE extracts by qualitatively monitoring for the phenolic compounds, which are formed in the injection port. When a presence of these compounds was indicated, quantitation was done from an injection of the derivatized extract. The dual extraction methods of SPE and liquid-liquid extraction ensured an acceptable recovery for all the analytes under study. This study offers a concise method for the analysis of a wide variety of the most commonly used pesticides at trace level.

Fig. 2 GCMS/SIM chromatogram of 1 μL injection of a standard mixture containing between .50 and 10 ng of each compound. The quantitation and the confirmation ions (m/z) are as follows.

Quantitative Analysis of LAS Using Continuous-Flow FAB Tandem Mass Spectrometry

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Indiana University, Bloomington, Indiana 47405

Introduction. Linear alkylbenzenesulfonates (LAS), the most common commercial
surfactants, are introduced into the environment at rates of approximately
300,000 tons/year. Clearly, understanding the environmental fates of these
compounds is important. LAS have been measured using liquid chromatography and
GC-MS after one or more derivitization steps (1-2). Our laboratory is using
continuous-flow fast-atom bombardment (CFFAB) tandem mass spectrometry to give
quantitative and qualitative information on LAS occurring in environmental
samples.

In the study of alkylbenzenesulfonates by CFFAB tandem-quadrupole mass spectrom-
etry, only one daughter ion was found containing an intact benzenesulfonate
moiety. This daughter ion is at m/z 183 for linear alkylbenzenesulfonates and
at m/z 197 for alkylbenzenesulfonates containing branched alkyl chains. See
Figure 1 for spectra and proposed fragmentation schemes.

We have developed a method for the analysis of LAS which takes advantage of this
behavior. By scanning the parent ions of the ethylene substituted benzenesulfo-
nate ion at m/z 183, LAS are selectively identified. Because of this selectivi-
ty, sample preparation is reduced to concentration of the analyte in liquid
samples, and extraction from solid samples followed by concentration of the
extract. In addition, since we use flow injection introduction of the sample to
the mass spectrometer, analysis times are reduced to as low as 2 minutes, allow-
ing much quicker throughput than chromatographic methods.

Experimental. For all experiments, a flow injection system was utilized,
consisting of an Isco μ-LC 500 syringe pump and a Valco CI4W injection valve
with a 0.5 μl injection volume. A mixture of 50% methanol, 45% water, 5% gly-
cerol was pumped at a flow rate of 5 μl/min into a VG Trio 3 triple quadrupole
mass spectrometer. The probe tip was held at 50 °C. Argon was used as the
collision cell gas at a pressure of 2.7 mtorr as measured by a Baratron capaci-
tance manometer, while the collision energy of the cell was set to 20 eV. The
commercial FAB source and continuous-flow FAB interface were modified slightly
to improve performance: A small cup was soldered to the source heating block
which allowed a sorbent pad to be placed beneath the probe tip and absorb excess
liquid and prevent sudden evaporation. In addition, new probe tips were made
which included a 1/16" diameter frit and a 0.5 mm deep notch to control the
stability and direction of the liquid flow across the tip.

Water samples were passed through a 0.5 μm filter and concentrated by pumping
them through a 4.6 mm X 25 cm column packed with Bondesil preparative grade SAX
material. LAS were eluted with 0.2 M HCl in methanol, and concentrated to 1 ml.
LAS in a sample from a primary treatment tank in an activated sludge treatment
plant were determined by filtering the sample, removing the analyte from the
solid material by sonication in methanol, filtering the solids again, and con-
centrating the supernatant to 1 ml by evaporation.

Results. The CFFAB parent-ion method gives consistent results over a wide range
of concentrations, as indicated in Figure 2. Response to LAS is linear over a
range from 10 ng to 1000 ng (Figure 3). Figure 4 shows the parent-ion spectrum
of an equimolar mixture of C₁₀ to C₁₂ LAS homologues, indicating that the system
response to each of these homologues is equal. Therefore, each LAS homologue in
a sample can be determined by integrating the signals of the parent ion corre-
sponding to LAS with a given chain length.
The method was applied to the analysis of LAS in an activated sludge treatment plant. Samples were taken from the plant influent, the primary treatment tank, and the river into which the effluent from the plant flows. Figure 5 shows the parent-ion spectrum of the primary treatment sample, indicating the presence of C_{10} to C_{13} LAS homologues. Figure 6 shows the total concentration of LAS at each location as well as the concentrations of the individual homologues. Concentrations were determined against an external calibration curve. Each of these analyses was done in duplicate; the total analysis time was 18 minutes.

Reduction of Azo Dyes to Aromatic Amines for Monitoring Dyes in Waste Streams

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North Carolina State University, Box 8302
Raleigh, NC 27695 USA

The limited availability of suitable analytical methods for the analysis of a wide variety of azo dyes hinders the ability to regulate their emission into the environment. Development of analytical methods for dyes is difficult due to the nonvolatility and thermal lability of dyes, the complexity of dye mixtures, and the variety of dye types (e.g., acids, solvents, disperses, etc.). The reduction of azo dyes to aromatic amines greatly simplifies the analytical problem since most of the aromatic amines (originating from all dye types) can be measured by one analytical technique. While reduction of azo dyes in solvent has been reported (1), the success of reduction of dyes in waste samples has been limited.

Three chemical procedures were evaluated for the reduction of azo dyes. The first reduction agent, tin chloride (SnCl₂), is important in the reduction cleavage of azo linkages in the presence of other easily reduced groups, such as a nitro group. The second method which involves sodium dithionate (Na₂S₂O₄) has been used to effect the reductive cleavage of water soluble azo dyes for decolorizing used dye baths. The third chemical procedure involved the in situ catalytic reduction of the azo dyes by hydrogen (H₂) in the presence of palladium (Pd) under the elevated temperature of a gas chromatographic injector.

The results observed from the reduction cleavage reaction of azo dyes are presented in Table 1. It was observed that the chemical reduction methods resulted in nearly 100% reduction of the azo bond to form the characteristic amines. Overall, the tin chloride method was a more powerful reducing agent, yielding a greater number of products. The in situ reduction of the azo dyes with H₂/Pd resulted in the formation of products consistent with the SnCl₂ and Na₂S₂O₄ reduction of the azo dyes, plus additional reduction products not previously observed.

These three reduction methods were evaluated on a dye containing wastewater sample. The dye was extracted with methylene chloride before undergoing reduction. Particle beam high performance liquid chromatography/mass spectrometry (HPLC/MS) analysis of the SnCl₂ and Na₂S₂O₄ reduced wastewater extracts indicated the presence of reduced, but unidentified compounds. Gas chromatography/mass spectrometry analysis of the wastewater using H₂/Pd produced reduction products consistent with azo dye structures. Table 2 shows a brief list of the HPLC/MS and GC/MS results.

Reduction methods permitted the detection of dyes that could yield aromatic amines under anaerobic degradation conditions that otherwise were not detected using standard MS techniques such as GC/MS or particle beam - HPLC/MS. While the identity of the dye could not always be postulated, their reduction products (aromatic amines) provided means to measure and possibly regulate azo dye wastes.

This work was supported in part by EPA Contract Number 68-02-4544.

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Table 1. Reduction Products Formed From Selected Dyes

<table>
<thead>
<tr>
<th>Dye</th>
<th>Reduction Products</th>
<th>SnCl₂ Reduction Identification by</th>
<th>Na₂S₂O₄ Reduction Identification by</th>
<th>Pd/H₂ Reduction Identification by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HPLC/MS</td>
<td>GC/MS</td>
<td>HPLC/MS</td>
</tr>
<tr>
<td><strong>Solvent Yellow 2</strong></td>
<td>• aniline</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>• N,N-diethyl-4-diaminobenzene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Schiff Red 24</strong></td>
<td>• 5-bromide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• 2,4-diaminobenzene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• 1-amino-2-naphthol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• 1-benzylanthrophenesin</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Disperse Red 11</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>• 4-amino</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• N,N-diethyl-4-diaminobenzene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• N,N-diethyl-4,6-diaminobenzene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Disperse Orange 25</strong></td>
<td>• 3-methoxy-4-hydroxyethyl-4-diaminobenzene</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>• 4-diaminobenzene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Disperse Black 5</strong></td>
<td>• 1,4-diaminobenzene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• 1,6-dinitro-4-hydrazobenzene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• N,N-diethyl-4-diaminobenzene</td>
<td></td>
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<tr>
<td><strong>Disperse Orange 37</strong></td>
<td>• 3,5-dichloro-4-nitroaniline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• 3-chloro-1,4-diaminobenzene</td>
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<tr>
<td><strong>Disperse Brown 11</strong></td>
<td>• 3,5-dichloro-4-nitroaniline</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>• 3-chloro-1,4-diaminobenzene</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>• 1,4-diaminobenzene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• 2-chloro-1,4-diaminobenzene</td>
<td></td>
<td></td>
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</tbody>
</table>

X = Reduction products identified by mass spectra.

Table 2. Reduction Products Detected by HPLC/MS and GC/MS from a Dye Containing Wastewater Sample

<table>
<thead>
<tr>
<th>Reduction Procedure</th>
<th>Number of Components Separated by HPLC/MS&lt;sup&gt;a&lt;/sup&gt;</th>
<th>GC/MS&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>SnCl₂/HCl</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Na₂S₂O₄</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Pd/H₂</td>
<td>6&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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</tbody>
</table>

<sup>a</sup>Number of new main reduction products separated by high performance liquid chromatography (HPLC) and gas chromatography (GC).

<sup>b</sup>Reduction products identified from their mass spectra: 2-chloro-1,4-diaminobenzene, 4-nitroaniline and 4-nitrodiaminobenzene isomer.
Mass Spectral Identification of Compounds Present in Toxic Fractions of a Dye Manufacturing Effluent

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INTRODUCTION

In response to the Water Quality Act of 1987 many state regulatory agencies require industries and municipalities to perform biomonitoring tests to determine the toxicity of their effluent to aquatic biota. Accordingly, the US Environmental Protection Agency has developed a Toxicity Identification Evaluation (TIE) protocol to guide the identification of toxicants in industrial and municipal effluent. This protocol is a series of fractionation procedures used to separate toxic and nontoxic components. Toxicity of the fractions is determined using acute tests with Ceriodaphnia dubia as the test organism. In this study we present a case history that uses the TIE protocol to identify/characterize the aquatic toxicity of effluents from a dye industry. The suspected contributors to effluent toxicity were chloride, which results from the use of sodium chloride to precipitate dyes, and some portion of the high organic content. Dyes, unreacted dye precursors, dye intermediates, surfactants, or byproducts from biological treatment were possible sources of the organic toxicity in the effluent.

METHODS

Toxicity testing of whole effluent samples and chemical fractions were performed using Ceriodaphnia dubia, an invertebrate, as the test species. Acute toxicity tests were performed, with lethality as the end point. Toxicity was measured in terms of an IC\textsubscript{50} or an ET\textsubscript{50}. The concentration at which 50% of exposed organisms die within a specified amount of time (in this case, 48 hours) is an IC\textsubscript{50} (g/L) and the time it takes for 50% of exposed organisms to die at a given concentration is an ET\textsubscript{50} (hours).

Samples of the effluent from the industrial waste treatment facility at the dye manufacturing plant were collected on January 9, 1990 (Sample 1), April 9, 1990 (Sample 2), and July 31, 1990 (Sample 3). The procedures outlined in the EPA TIE Phase I and II documents were followed in the examination of the three samples. The EPA TIE Phase I procedures are used to characterize effluent toxicity as metals, volatile compounds, nonpolar organic compounds, particulates, oxidants, or ammonia. Those tests that were successful in reducing toxicity pointed to specific classes of chemicals as causative agents.

To determine the contribution and identity of the nonpolar compounds to effluent toxicity, EPA TIE Phase II procedures were followed. Solid phase extraction (SPE) with a C\textsubscript{18} stationary phase and reverse phase HPLC fractionation were used to separate toxic and nontoxic chemicals in the effluent samples. Methanol, the solvent used in the fractionation procedures, is not toxic to C. dubia at or below 1.5%. Controls were taken through the fractionation procedures in order to account for artifactual toxicity introduced by the methods.

High-resolution gas chromatography/mass spectrometry (HRGC/MS) was used to identify components of the final toxic fractions from the Phase II procedures. The gas chromatographic conditions were an initial temperature of 50°C for 1 minute, followed by a temperature increase to 125°C at a rate of 5°C/min, the rate then was increased to 10°C/min and the temperature was brought to 300°C and held for 10 minutes. The GC column was a J&W Scientific DBS column, 30 m, 0.25 mm i.d., 0.25 μm film. A Hewlett Packard Model 5890 Series II gas chromatograph was interfaced with a VG model 70-250SBQ double-focusing mass spectrometer. Interpretation of electron ionization (EI) mass spectra provided information about the unknown components of the toxic fractions. Chemical ionization (CI) using methane, isobutane, or ammonia as the reagent gas was useful in verifying the molecular ions of some of the compounds identified by EI/MS. Accurate mass measurements from GC separation of toxic fractions of Samples 1 and 3 were performed. Identifications were verified by standards obtained from Aldrich Chemical Company or by finding matching spectra in the National Institute of Standards and Technology computer library of the Eight Peak Index of Mass Spectra.

RESULTS AND DISCUSSION

The C\textsubscript{18} SPE column was effective in removing a portion of the effluent toxicity from all three samples. Metals, oxidants, ammonia, particulates, and polar organic compounds were not the cause of the remaining toxicity. Measurement of the concentration of chloride in the whole effluent and comparison of the resulting toxicity to the measured toxicity of chloride...
showed that the toxicity that could not be extracted by the C<sub>18</sub> SPE column resulted from chloride. The concentration of chloride in both Samples 2 and 3 was 4.6 g/L. Chloride accounted for approximately 1/3 of the toxicity in Sample 2 and 1/2 of the toxicity in Sample 3. The remaining toxicity was caused by nonpolar organic compounds which could be fractionated by C<sub>18</sub> SPE and HPLC.

The compounds identified in toxic fractions where of three types: azo dyes, non-dye aromatics, and compounds related to 2,4,7,9-tetramethyl-5-decyne-4,7-diol (TMDD). Standards of some of the identified chemicals were tested for toxicity to <i>C. dubia</i>. The largest peak in the toxic fractions of the effluent samples was TMDD. Isomers and byproducts of TMDD were also identified in the fractions. A spectrum of TMDD is presented in Figure 1. The molecular ion of this compound is not observed due to the facile loss of a butyl group. 2,4,7,9-tetramethyl-5-decyne-4,7-diol is a component of Surfynol 104E, which is used at the plant to control foaming and to neutralize soda ash.

Twelve small azo dyes, with molecular weights less than 250 amu, were identified in the toxic fractions, only two of which were found in more than one fraction. Azo dye spectra were easily recognizable due to their consistent fragmentation patterns. As illustrated by the EI spectrum of 4-phenylazophenol in Figure 2, a fairly strong molecular ion is observed for azo dyes. The molecular ion initially loses a phenyl group and its substituents to form [M-PhX]<sup>-</sup>. This is followed by the loss of the azo group (N=N) to form the base peak. Subsequently the substituents of the remaining phenyl group are lost to form a peak at m/z=77 or m/z=76 for [C<sub>6</sub>H<sub>4</sub>]<sup>-</sup> or [C<sub>6</sub>H<sub>5</sub>]<sup>-</sup>, respectively.

**CONCLUSIONS**

Effluent acute aquatic toxicity was determined to be caused by chloride and nonpolar organics. No organic component of the three effluent samples was found to be solely responsible for organic toxicity, rather toxicity was thought to result from the interaction of several toxic chemicals present in low concentrations. The presence of azo dyes could be easily determined by GC/MS, due to their characteristic fragmentation pattern produced by electron ionization.

**REFERENCES**


ANALYSIS OF CHELATORS AND CHELATOR FRAGMENTS IN MIXED HAZARDOUS WASTES BY DERIVATIZATION GC/MS

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INTRODUCTION

Permanent waste disposal technologies for mixed hazardous wastes are currently being developed for implementation in the near future. The characterization of mixed hazardous waste is one criterion that needs to be addressed before the waste can be permanently disposed. Since chelators and their degradation products are often major constituents in mixed hazardous waste and their analysis is not amenable to gas chromatography (GC) without prior derivatization, alternative analysis methods need to be utilized. Previously, we have examined and reported on derivatization of chelators in mixed hazardous waste by methylation with BF₃/methanol followed by GC and gas chromatography/mass spectrometry (GC/MS).¹² The BF₃/methanol derivatization may not be applicable to all chelators, and therefore alternative methods such as silylation, diazomethane, and butyl esterification need to be compared. In this study, we derivatized ethylenediaminetetraacetic acid (EDTA), hydroxyethylethylenediaminetriacetic acid (HEDTA), ethylenediaminetriacetic acid (ED₃A), and iminodiacetic acid (IDA), with subsequent GC/MS analysis.

EXPERIMENTAL

Methylation by diazomethane, silylation by BSTFA, butyl esterification by butanol/HCl, and methylation by BF₃/methanol were achieved for chelator standards obtained from Aldrich and independent sources. GC/MS analysis was obtained on a Hewlett Packard HP 5988 GC/MS system. A fused silica DB5, 30 m x 0.25 mm i.d. (0.25-µm film thickness) column was used in the splitless injection mode. The oven program consisted of 40°C for 1 min followed by 8°C/min to 300°C. The mass spectrometer was scanned from 40-600 amu with 70-eV electron impact ionization.

RESULTS / CONCLUSIONS

Diazomethane methylation: This reaction occurs at ambient temperature and is a slow reaction for chelators; often it will take several hours to complete the reaction. The mass spectrum of IDA, when derivatized with excess diazomethane, shows a peak at m/z 175; however, the methyl ester has a molecular weight of 161. These results indicate an example of multiple methylation that often takes place with the use of diazomethane.⁴ For ED₃A and HEDTA, a lactam and lactone were observed.

BSTFA silylation: The parent ion typically fragments severely for chelators, which can make mass spectral interpretation difficult at times. Silylation occurs at the carboxylic acid groups and the hydroxy groups of chelators.

BF₃/methanol methylation: Chelators were methylated at the carboxylic site and not at the hydroxy sites. For ED₃A (MW 234) derivatized with boron trifluoride/methanol, one observes a peak at m/z 244 in the mass spectrum, which indicates the formation of a lactam. HEDTA, derivatized with boron trifluoride/methanol, is identified by the formation of a lactone at m/z 288. BF₃/methanol requires heating the sample to 100°C, which has the potential to thermally degrade the component of interest.

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REFERENCES


Figure 1. Mass spectra of derivatized chelators. (A) diazomethane of IDA, (B) BSTFA of HEDTA, (C) butanol/HCl of EDTA, and (D) BF₃/methanol of EDTA.

** This work was supported by the U.S. Department of Energy under Contract DE-AC06-76RLO 1830. Pacific Northwest Laboratory is operated by Battelle Memorial Institute.
MULTISPECTRAL IDENTIFICATION OF CHLOROALKYL PHOSPHATE FIRE RETARDANTS IN AN INDUSTRIAL EFFLUENT


Introduction

Under the Clean Water Act Amendments of 1987, the U.S. Environmental Protection Agency continually reviews the occurrence of potentially harmful chemicals in industrial waste waters. Selected waste effluents are analyzed to identify chemicals that have not been identified previously either because they were not present or because improvements in identification methods permit their detection and identification for the first time. Recently, organic extracts of samples taken from several plants manufacturing organic chemicals were analyzed using multispectral identification techniques not generally applied previously. Among the plants sampled was a plant that manufactures fire retardants.

Alkyl, haloalkyl, and aryl phosphates have been widely used as flame retardants in a variety of products. The more common aryl phosphates are found in plasticizers, lubricants, and hydraulic fluid. The chloroalkyl phosphates have been used both in fabrics and in insulation materials. Many studies have been performed to determine the toxicity of these phosphates, as warranted by their widespread use in fabrics, plasticizers, hydraulic fluids, and lubricants. The results showed phosphates to be absorbed into the body and to be toxic to fish.

We identified 13 alkyl and chloroalkyl phosphates and also demonstrated how multispectral analysis can be used to identify organic compounds in environmental samples. Multispectral techniques--gas chromatography coupled with high and low resolution electron-impact mass spectrometry (GC/EI-MS), high and low resolution chemical ionization mass spectrometry (GC/CI-MS), and Fourier transform infrared spectroscopy (GC/FT-IR)--provided structural information that permitted the identification of these alkyl phosphates.

Experimental

Samples were collected from the effluent of a plant that manufactures fire retardant chemicals and were extracted according to EPA Method 1625C. GC/MS analyses were performed on a VG 70-SEQ high resolution hybrid mass spectrometer, equipped with the VAX-based OPUS data system and a Hewlett Packard Model 5890A gas chromatograph. The GC/FT-IR analyses were performed using a Digilab Model FTS-60 FT-IR spectrometer equipped with a Digilab Model 3200 FT-IR workstation and a Hewlett Packard Model 5890 gas chromatograph with a cool on-column injector.

Results and Discussion

The conventional method for identifying organic compounds found in the environment -- low resolution EI-MS with library database matching -- was only partially beneficial to the identification of the phosphates found in this industrial effluent. Only 4 of the 13 phosphates gave good library database matches. Therefore, techniques other than conventional low resolution EI-MS with database matching were required. One example of how we used multispectral techniques to identify these compounds was the identification of bis(2-chloroethyl)-2-(2-chloroethoxy)ethyl phosphate (Compound 8); its low resolution mass spectrum exhibited many of the same ions seen in the mass spectrum of tris(2-chloroethyl)phosphate (Compound 4), indicating that it was a phosphate with a similar structure. However, this compound showed a molecular weight of 328 by CI-MS. It was found by high resolution CI to contain an additional oxygen atom and to have an empirical formula of C₈H₁₅O₃Cl₂P. There are 3 primary scissions evident from the EI spectrum for Compound 8: the loss of Cl, CH₂Cl, and OC₂H₄Cl.
Infrared spectroscopy provided the remaining necessary pieces of information. The additional oxygen atom would most likely be present as an ether, a carbonyl, or an alcohol functionality. There was no indication in the infrared spectrum of either C=O or O-H stretching peaks (both of which are easily detected in the infrared at about 1650-1850 cm\(^{-1}\) and 3500-3700 cm\(^{-1}\), respectively). Unlike OH and C=O, ether linkages, which absorb at about 1100 cm\(^{-1}\), cannot always be unequivocally assigned due to absorbances of many other vibrations in this region. Also in comparing the IR spectrum of Compound 8 to that of Compound 4, we observed a shoulder on the intense P-O-C stretching peak at about 1135 cm\(^{-1}\) for Compound 8, which is not present in the spectrum of Compound 4. This indicates the presence of an ether linkage in the structure of Compound 8. However, in light of the empirical formula provided by high resolution MS, the most conclusive evidence for an ether linkage in this structure is the absence of OH and C=O IR absorbances. The high resolution MS and infrared data, coupled with the similarity of the low resolution mass spectrum and IR spectrum to that of tris(2-chloroethyl)phosphate, indicate Compound 8 is bis(2-chloroethyl)-2-(2-chloroethoxy)ethyl phosphate.

The 13 identified compounds were dimethylmethyl phosphonate, trimethyl phosphate, triethyl phosphate, tris(2-chloroethyl)phosphate, tris(1-chloro-2-propyl)phosphate, bis(1-chloro-2-propyl)(3-chloro-1-propyl)phosphate, bis(3-chloro-1-propyl)(1-chloro-2-propyl)phosphate, bis(2-chloroethyl)-2-(2-chloroethoxy)ethyl phosphate, bis(1-chloro-2-propyl)-3-(3-chloropropoxy)propyl phosphate, (1-chloro-2-propyl)(3-chloro-1-propyl)-3-(3-chloropropoxy)propyl phosphate, (1,3-dichloro-2-propyl)phosphate, bis(1,3-dichloro-2-propyl)(2,3-dichloro-1-propyl)phosphate, and 1,2-bis[bis(2-chloroethyl)-phosphato]ethane.
CHEMICAL IONIZATION MASS SPECTROMETRY OF O-ETHYL S-[2-DIMETHYLAMINOETHYL] METHYLPHOSPHONOTHIOATE AND ITS DEGRADATION PRODUCTS
Dennis Rohrbaugh, U.S. Army Chemical RD&E Center, APG, MD 21010-5423

Recent multinational interest in chemical weapon treaty verification has renewed interest in the detection of chemical warfare agents and their degradation products, particularly organophosphorus compounds. Many of these compounds are difficult to analyze by conventional electron ionization methods because extensive fragmentation results in little or no molecular ion information. In addition, compounds containing the dialkylaminoethyl group have similar EI spectra which are difficult to distinguish. In this study we apply capillary GC/MS using methane as the reagent gas to detect and identify 18 products resulting from the degradation of O-ethyl S-[2-dimethylaminoethyl] methylphosphonothioate. This technique provides the valuable molecular ion and fragmentation information necessary to identify these compounds.

Spectra were obtained on a Finnigan 5100 GC/MS equipped with a fused silica 15m x 0.25mm id SE-54 capillary column. Injection temp was 200°C and the oven was programmed from 60°C to 260°C at 10°C/min. CI reagent gas was methane (0.6 Torr). The mass range was scanned from 58 to 450 amu at 1 scan/sec. A comparison of EI and CI spectra for the phosphonothioate in Figure 1 illustrates the usefulness of CI for identification of these compounds. Fragmentation structures are shown in Figure 2.

Products were identified for storage under both inert and atmospheric conditions. The total ion chromatogram obtained for the sample under an inert atmosphere (nitrogen) is shown in Figure 3. The identification of each of the 18 compounds and relative quantities detected under both storage conditions are listed in Table 1. Under inert storage conditions degradation occurs primarily to a mixture of pyrophosphonates, thiolamines and phosphonothiolates containing 1, 2 and 3 sulfur atoms. Exposure to the atmosphere results in considerable oxidation to disulfides.
TABLE 1. O-ETHYL S-[2-DIMETHYLAMINOETHYL] METHYLPHOSPHONOTHIOATE DEGRADATION PRODUCTS OBSERVED DURING STORAGE UNDER INERT AND ATMOSPHERIC CONDITIONS

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ANALYSIS OF SAMPLES FOR CHEMICAL WARFARE AGENTS: CANADIAN CONTRIBUTION TO A UNITED NATIONS VERIFICATION EXERCISE

* Paul A. D’Agostino and Lionel R. Provost, Defence Research Establishment Suffield, P.O. Box 4000, Medicine Hat, Alberta, Canada, T1A 8K6.

The possible entry into force of a Chemical Weapons Convention (CWC) has prompted many countries to consider the difficulties involved in ensuring compliance by all participating nations. Compliance monitoring will be required in the verification of alleged use, the storage and destruction of chemical weapons stocks and to ensure that industrial sites are not illegally producing chemical warfare agents. The United Nations Conference on Disarmament, realizing that these scenarios would require the development and use of analytical techniques to ensure compliance, formed a multinational Technical Group on Instrumentation to address the analytical challenges confronting signatory nations. Nations involved in the Technical Group on Instrumentation recently participated in a round robin analytical exercise designed to evaluate the capabilities and analytical approaches of the laboratories. Samples, taken from the feedstock, product and waste streams within a chemical industry utilizing compounds that would be scheduled by a CWC, were circulated to sixteen National Laboratories for analysis. Participating laboratories were asked to document their methods and report the presence of chemical warfare agents, their degradation products or other scheduled compounds. Canada participated in this and a prior exercise (1) and utilized in-house methods for sample clean-up and capillary column GC-MS analysis (2,3).

Twenty-nine cotton swab, Tenax, XAD resin, charcoal, aqueous and blank samples were received by the participating laboratories following inspection of an industrial plant. The samples were extracted with 1 to 10 mL dichloromethane (cotton swabs and charcoal samples) or hexane (Tenax and XAD resin samples) in an ultrasonic bath for 2 to 5 minutes. Aqueous samples were extracted with 2 x 1 mL hexane. Sample extracts were screened by capillary column GC-FID and GC-FPD (phosphorus mode) and scheduled compounds were confirmed by capillary column GC-MS under the following electron impact (El) and ammonia chemical ionization (CI) conditions:

**GC**: 15 m x 0.32 mm ID DB-1701; 40°C (2 min) 10°C/min 280°C (4 min); on-column injection at 40°C.
**MS**: VG 70/70E; 6 kV; 500 to 40 u (0.75 sec/decade; 0.25 sec interScan delay); El (70 eV, 200°C, 100 µA, 10³ Torr); CI (50 eV, 110-130°C, 500 µA, 2x10⁻² Torr NH₃).

Degradation products, due to hydrolysis of scheduled organophosphorus compounds, were confirmed following trimethylsilylation (TMS) of the actual sample medium or extracts of the samples. Analysis of the provided samples clearly indicated that the site was producing the organophosphorus pesticide, Dichlorvos. Table I lists the compounds, including those scheduled under the proposed CWC, found in the provided samples. Four of the scheduled compounds, trimethyl phosphate, dimethyl phosphate, dimethyl methylphosphonate and methylyphosphonic acid, would be expected at an industrial plant producing Dichlorvos. Dioctyl methylphosphonate, a scheduled compound, could not be explained at a Dichlorvos production site and its presence would create concern for a CWC Inspectorate. Figure 1 illustrates the GC-MS chromatogram of the hexane extract of an aqueous sample. Dioctyl methylphosphonates were tentatively identified in this extract and several other sample extracts by interpretation of the acquired El and ammonia CI data (Figure 2). The El mass spectra of all the dioctyl methylphosphonates exhibited a base ion at m/z 97 due to [(HO)₂P(CH₂)₁₂]⁻ and an ion at m/z 209 due to (M+H)⁺. Molecular ion information, critical to structural assignment, was absent in the El mass spectrum, but was evident during ammonia CI analysis. The CI mass spectra of the dioctyl methylphosphonates were dominated by (M+H)⁺ and (M+NH₄⁺) ions at m/z 321 and 338 respectively. CI fragmentation ions due to loss of C₆H₄OH from the pseudo-molecular were observed at m/z 209 and 226. Tentative identification of dioctyl methylphosphonates was confirmed by synthesis of several isomers.

Canada identified more than 20 compounds in the samples, including 5 compounds scheduled under the proposed CWC. Thirteen of the 16 participating National Laboratories (including Canada) identified the presence of the organophosphorus pesticide, Dichlorvos, and detected the scheduled compounds, trimethyl phosphate, dimethyl phosphate and dimethyl methylphosphonate. Nine of the 16 laboratories (including Canada) identified the scheduled degradation product, methylyphosphonic acid. These organophosphorus compounds could not be explained at a Dichlorvos plant and would not raise compliance monitoring concerns under the proposed CWC. Only 5 of the 16 participating National Laboratories (including Canada) identified the presence of dioctyl methylphosphonates. These scheduled compounds would not be associated with Dichlorvos production and would not raise compliance monitoring concerns under the proposed CWC. Two of these 5 laboratories also detected traces of octyl methylyphosphonofluoridate.

**References**

### Table 1

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<td>186</td>
<td>Chloroethyl dimethyl phosphates</td>
</tr>
<tr>
<td>15&lt;sup&gt;*&lt;/sup&gt;</td>
<td>220</td>
<td>Dichlorvos</td>
</tr>
<tr>
<td>16</td>
<td>320</td>
<td>Dioctyl methylphosphonates</td>
</tr>
<tr>
<td>17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>198</td>
<td>TMS derivative of dimethyl phosphoric acid</td>
</tr>
<tr>
<td>19</td>
<td>266</td>
<td>tri-TMS derivative of phosphorous acid</td>
</tr>
<tr>
<td>20</td>
<td>226</td>
<td>di-TMS derivative of hydrogen phosphonic acid</td>
</tr>
<tr>
<td>21</td>
<td>256</td>
<td>di-TMS derivative of methyl phosphonic acid</td>
</tr>
</tbody>
</table>

<sup>a</sup> Spiked into samples.  <sup>*</sup> CWC scheduled compound.

### Figure 1

GC-MS (EI) of hexane extract of a Waste (WS2)

[Graph showing GC-MS data with peaks labeled 10: C₆ Alcohols, 17: Dioctyl methylphosphonates; 19m DB-1701; 40°C (2 min) 10°C/min 280°C (4 min)].

### Figure 2

a) El and b) Ammonia CI mass spectra of a typical Dioctyl methylphosphonate (tentative Id)

[Graph showing mass spectra with peaks labeled (C₈H₁₇O)₂-P-CH₃ and other molecular ions].
EVALUATION OF AIR CLEANER EFFICIENCY WITH AN APCI MASS SPECTROMETER

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Bowman Gray Technical Center
Winston-Salem, NC 27102 USA

Some have suggested that exposure to environmental tobacco smoke (ETS) in occupational environments should be limited. One way to minimize the impact of ETS on indoor air quality would be the use of effective air cleaners. The results reported here are a subset of a larger experiment to gauge the effectiveness of one prototype and two commercial air cleaners. ETS was generated in an 18-m³ controlled environment test chamber. A SCIEX® TAGA® 6000 mass spectrometer was used to monitor the air concentrations of nicotine, pyridine, picoline, ethylpyridine, and ethenylpyridine in real time. Experimental data were analyzed to determine whether use of the air cleaners resulted in significant reductions of these analytes in the chamber and to determine the effective clean air delivery rate (CADR) of each air cleaner.

The chamber, analyzers, calibration, and data analysis have been described elsewhere. Temperature and relative humidity were controlled at 22°C and 50%. The chamber was ventilated at a rate of 6 air changes per hour. Five runs were performed with each air cleaner and ETS. The following air cleaners were evaluated: OZNI-commercial ozone/negative ion generator, unit was adjusted so that ozone concentrations in the chamber did not exceed 50 ppm (half of ozone TLV); ESP-commercial electrostatic precipitator with minimal charcoal filtration; COMB-prototype unit with physical filtration, UV ozone generation, and charcoal filtration. Environmental tobacco smoke was initially generated by sequentially machine smoking two pairs of University of Kentucky 1R4F reference cigarettes. Then, individual cigarettes were sequentially machine smoked until the end of the experiment. The smoking was preceded by a twelve-minute background determination.

The average real-time pyridine concentration in the chamber during each test is depicted in Figure 1. Similar response was observed for each substituted pyridine measured. Concentrations appear to be the highest with the OZNI cleaner on. This may be due to the formation of nicotine pyrolysis products in the ozone generation discharge. Although the ESP was highly efficient at removing particles, it was not designed for the effective removal of vapor phase compounds. The COMB air cleaner typically removed =50% of the target compounds. This was probably due to the adsorption of gas phase components onto the charcoal bed.

A one-way ANOVA was performed on results for each analyte by air cleaner type. Following ANOVA, a Bonferroni-adjusted multiple comparison test was performed to determine whether differences among air cleaners were significant at the p<.05 level. For the removal of pyridine, picoline, and ethenylpyridine: Neither OZNI nor ESP significantly reduced chamber concentrations; concentrations were significantly higher for OZNI compared to ESP; and COMB significantly reduced concentrations of these compounds in the chamber. For the removal of ethylpyridine: no significant differences were observed between the no air cleaner, OZNI, or ESP conditions; COMB significantly reduced concentrations of this compound relative to the other air cleaners and the no air cleaner condition. For the removal of nicotine: there was no significant difference in concentrations with the OZNI and no air cleaner conditions; concentrations were not significantly different with the OZNI, ESP, or COMB air cleaners in operation; both the ESP and COMB air cleaners significantly reduced nicotine concentrations relative to the no air cleaner condition.
CADR is the equivalent volume of fresh air provided by an air cleaner per unit time. An air cleaner with a given CADR has the same effect on contaminant concentration as diluting the contaminant by ventilation with fresh air. The CADR is useful for comparing the suitability of different air cleaners for removing indoor air contaminants. The greater the CADR, the more effective the air cleaner is. Unfortunately, all compounds in indoor air may not be removed with the same efficiency by an air cleaner. It then becomes important to know the CADR of the air cleaner with respect to particular compounds of interest.

Air cleaner rate constants were determined from "concentration vs. time data" by application of a simple mathematical model of chamber dynamics. The model assumes a first-order removal mechanism and a zero-order (constant) generation rate. It was assumed that effective equilibrium had been reached during the final 10 minutes of each run. CADR's were subsequently calculated from the model. The table below shows airflows and CADR's for each compound measured with the TAGA®. Negative numbers correspond to elevated concentrations relative to the no air cleaner condition.

<table>
<thead>
<tr>
<th>Air Cleaner</th>
<th>Flow Rate CFM</th>
<th>CADR for Each Compound, CFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>OZNI</td>
<td>150</td>
<td>Nicotine -9, Pyridine -5, Picolin -8, Ethylpyridine -9, Ethenylpyridine -9</td>
</tr>
<tr>
<td>ESP</td>
<td>160</td>
<td>Nicotine 22, Pyridine 7, Picolin 5, Ethylpyridine 2, Ethenylpyridine 4</td>
</tr>
<tr>
<td>COMB</td>
<td>167</td>
<td>Nicotine 25, Pyridine 65, Picolin 53, Ethylpyridine 41, Ethenylpyridine 43</td>
</tr>
</tbody>
</table>

Conclusions

Real-time data obtained with the TAGA® can be used to determine decay kinetics of compounds in indoor air. Air cleaners designed for the removal of particles from indoor air may be less effective at removing gas phase compounds. Ideally, an air cleaner should combine methods for the removal of both gaseous and particulate phase compounds.

References


Acknowledgements

The work done by Mr. Dave Heavner and Ms. Patricia (T.C.) DeLuca in the organization and execution of the ETS chamber experiments is gratefully acknowledged. Thanks are also due to Ms. Barbara Collie for her help with the daily operation of the TAGA®.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

THERMAL STUDY OF SOLANESOL BY ON-LINE THERMOGRAVIMETRY/ GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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R. J. Reynolds Tobacco Company, Bowman Gray Technical Center, Winston-Salem, NC 27102

Solanesol (3,7,11,15,19,23,27,31,35-nonamethyl-2,6,10,14,18,22,26,30,34-hexatriacontanonaene-l-ol) is the most abundant terpenoid in tobacco and is a major component of the lipid fraction. Lipid constituents of tobacco or their thermal degradation products are related to the flavor and aroma of tobacco smoke.

An on-line thermogravimetry/gas chromatography/mass spectrometry (TG/GC/MS) system was designed, constructed and used to study the stability, weight loss, and the thermal degradation products of solanesol. The system permits evolution of thermal degradation products of solanesol by the TG, separation of the volatile thermal degradation products by the GC, and identification of the volatiles by the MS. The on-line TG/GC/MS system employed a Du Pont 951 thermogravimetric analyzer equipped with a 9000 thermal analysis system interfaced to a Hewlett-Packard 5890 GC/5970 MSD system. The TG/GC interface consisted of a heated transfer line, an adsorbent trap packed with Tenax and a six-way valve. Solanesol was heated and purged with air at a flow rate of 30 ml/min during the heating and purging process. The TG temperature was programmed from 50°C to 900°C at 10°C/min. The volatile compounds generated in the TG were swept into the adsorbent trap. The purge gas, after passing through the trap at room temperature, was vented to the atmosphere. After the heating and purging process, the trap was rapidly heated to 200°C and the volatile compounds in the trap were swept into the GC/MS system by helium at a flow rate of 35 ml/min. The volatile compounds were separated by GC. A 60 m X 0.25 mm ID DB-1701 (0.25 μm film thickness) fused silica capillary column was used for compound separation. The GC injector temperature was 250°C. Helium was used as the carrier gas. The column head pressure was 25 psi. The column temperature was held at 35°C for 5 minutes and then programmed to 220°C at 3°C/min. An open-split GC/MS interface was used with the temperature set at 275°C. The mass spectrometer was operated at 70 eV in the EI mode. The ion source temperature was maintained at 250°C. The mass range was scanned from 29-500 amu. The volatile compounds were identified by mass spectra. The thermogram of solanesol depicted a curve with two plateaus showing sample weight loss as a function of increasing temperature. The first decomposition occurred over a temperature range of 237°C-389°C with a total weight loss of 87% and a maximum decomposition rate at 320°C. The second degradation occurred between 389°C-496°C, and involved a further 13% weight loss with a slower decomposition rate. Solanesol decomposition was complete at 496°C. Thus, a temperature profile of stability and weight loss from solanesol could be obtained. The total ion chromatogram showed that a great number of compounds were thermally degraded from solanesol under the condition studied. The volatile degradation products were identified as aliphatic ketones, furan derivatives, aliphatic hydrocarbons, aromatic hydrocarbons, ethanone derivatives, aliphatic aldehydes, aliphatic alcohols, 2-cyclopenten-1-one derivatives, 2-cyclohexen-1-one derivatives, and phenol derivatives. Compounds known to contribute to the flavor and aroma of tobacco smoke are identified as acetic acid, acetoin, limonene, linalool oxide, nonanal, 2-methylbenzaldehyde, 2,6-dimethyl phenol, citral, farnesal, farnesol, and methyl oleate.
Removal of Salt Interference from FAB Spectra

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Introduction

Although the presence of salts is sometimes helpful in identifying molecular species in FAB mass spectra, their presence can often be deleterious. Cationization of matrix peaks adds to the chemical noise in spectra and this may completely mask less intense analyte peaks. Also, cationization spreads the analyte signal amongst a number of cationized species. Furthermore, large salt concentrations can completely suppress analyte ionization. This poster deals with the limitation of salts in the FAB experiment by revealing a method of sample washing for the removal of the water soluble impurities. We modified the method used by Beavis and Chait\textsuperscript{1} to remove salt interference from matrix-assisted laser desorption samples. Our method involves a fast, cold, aqueous extraction of the sample on the FAB target before the addition of matrix.

Wash Method

The sample is dissolved in a high vapor pressure solvent, typically methanol containing 0.1\% TFA, to achieve a concentration of about 1mM. A few uL of the solution is applied to the FAB probe with a gold tip and the solvent is allowed to evaporate. The probe tip is then immersed in an ice water bath (distilled water) for 1 - 5 seconds depending on the degree of salt contamination. The water remaining on the probe after withdrawal from the ice bath is removed by placing the probe in the insertion lock of the mass spectrometer and allowing the water to be pumped away. After taking the probe back out of the insertion lock, about 1 uL of the appropriate matrix is applied to the dried sample. The sample is now ready for analysis.

FABMS

FAB mass spectra were measured on a VG ZAB2-SE mass spectrometer using a cesium ion gun (12 kV) to provide energetic particles for desorption. Data acquisition was accomplished by the OPUS data system (version 1.5f). Instrument parameters were held constant, eg. multiplier gain, during experiments so that the only variable was the washing step.

Results and Discussion

Fig. 1a. shows the FAB spectrum of the products of reaction of the antibiotic daptomycin, MW 1619, with aqueous methanolic sodium cyanoborohydride. The submitter of the sample had previously attempted to desalt it. Daptomycin is an N-acylated peptide antibiotic containing a macrocyclic lactone. On the basis of the data in Fig. 2a. the submitter concluded that the ion at m/z 1642 was due to hydrolysis of the lactone ring and the concomitant reduction of two sites within the molecule (1619 + 18 + 2x2). Some of the peaks at lower mass were assigned to expected peptide bond cleavages of the new protonated molecule.

Fig. 1b. shows the FAB spectrum of the washed sample. It was now clear that two products had resulted from the reaction, unreacted daptomycin (MH+ = 1620) and a compound with MW 1651. The compound of MW 1651 was probably due to the addition of a mole of methanol, presumably through the methanolysis of the lactone. The peaks at m/z 563 + n(58) in spectrum 1a. were due to the antifoam agent polypropylene glycol, which was coincidently removed with the salts during washing.

The concentration of salt impurity in a sample can influence the washing time needed for optimum results. Fig 2a. shows the FAB spectrum of a peptide submitted to our laboratory. The data indicate a MW of approximately 6581 d. while a peak m/z 3294.3 could be interpreted as arising from a doubly-charged molecule with MW 6586.6 d. The spectrum of the sample obtained after a 2 sec. wash in Fig. 2b. A series of peaks spaced approximately 22 d. in the region of m/z 6500 indicate a MW of 6475.3 d., although a less well-defined peak at 6450.5 d. might suggest that 6476.3 d. is MNa+ rather than MH+. Washing the sample for 4 sec. removed this ambiguity. The spectrum of this washed sample, Fig. 2c., shows no evidence of cationization and clearly indicates a MW of 6475.3 d. measured from MH+ and 6477.0 d. measured from MH2++.
Conclusion

The washing technique is very useful although it is simple in theory and easy in practice. While probably not applicable to all classes of compounds, it can solve the difficult problem of water-soluble contaminants in FAB samples, especially with large biomolecules and mixtures.

As far as characterization of this technique, we have found that washing samples result in negligible losses of ion signal for many samples. In some cases where the ion signal is spread over the protonated molecule and sodium adduct ions, washing proved to increase absolute intensity of the protonated molecule.

Also, washing times for samples depend on the degree of salt contamination. In most cases washing for 1-2 sec. is all that is needed. However, some samples with unusually large amounts of salt may take on the order of 5 sec. for the salt impurities to be fully dissolved into the cold water.

Characterization of Organic Constituents of Produced Water and Marine Sediments Using GC/MS

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University of California, Davis, CA 95616
²Institute of Marine Sciences
University of California, Santa Cruz, CA 95064

Primary source water pollutants can have direct effects on aquatic populations. Produced water, the crude oil recovery processed aqueous effluent from offshore reservoirs, is usually piped directly into the ocean some distance from land.

Objectives

Biomarker analysis is widely used by the petroleum industry to study geochemical events leading to petroleum formation, but these techniques have not been brought to bear to determine sources of pollutants. It has been difficult to distinguish hydrocarbon pollutants that occur as a result of human activity from those that are due to natural forces such as oil seeps. Fortunately, the source of crude oil and the extent of its degradation can be determined from fingerprints of biomarker compounds, and GC/MS is the best method available for the task.

GC/MS Results

This project aims to develop and apply GC/MS analytical methods to quantify organic molecular indicators (biomarkers) of the amount of crude oil pollution, its sources, and the extent to which these pollutants have been weathered by environmental forces. The techniques we are developing will be applied to analyze for biomarker compounds in produced water, sediments, and biota to distinguish pollution from discharges of produced water versus discharges from other sources of petroleum products. The total ion chromatograms (TIC) (Figures 1 and 2) for the offshore crude oil/sea water emulsion and the west district sediment sample, down current from the produced water effluent pipe. The crude oil shows the characteristic n-alkane series while the sediment sample displays a weathered unresolved hydrocarbon pattern.

Figure 3 shows the reconstructed ion chromatograms (RIC) of the produced water effluent for the characteristic ion of triaromatic steranes, a major biomarker constituent of California source crudes (1). The pattern is identical to the source crude and similar to the sediment sample. Natural petroleum seeps in the Santa Barbara Channel have contributed to the observed biomarker pattern similarly.

The results indicate that the efficacy of current petroleum extraction methods, especially removal of branched chain and aromatic compounds, from the crude oil/sea water emulsion is suspect.

Acknowledgement

This work has been supported by the University of California Toxic Substances Teaching and Research Program in Coastal Toxicology.

References

Figure 1. TIC of inlet crude oil/sea water emulsion.

Figure 2. TIC of west district (down current) outfall pipe sediment site.

Figure 3. RIC of m/z 231 of triaromatic steranes of produced water effluent.
DISTRIBUTION OF PETROLEUM PRODUCTS WITH RESPECT TO BOATING ACTIVITY IN A RESERVOIR

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Environmental Engineering and Environmental Sciences Division
Virginia Tech, Blacksburg, VA 24061-0246

INTRODUCTION
The Occoquan Reservoir, located in Northern Virginia near Washington, D.C., serves as a water supply and recreational facility. The reservoir currently has three marinas—Bull Run, Fountainhead and Lake Ridge—and restricts the use of motor boats to engines of less than 10 HP. There is some concern that boating activity may contribute raw and combusted petroleum chemicals to the reservoir water and sediment.

This research was conducted to determine: 1) the concentrations of polycyclic aromatic hydrocarbons (PAHs) in water and sediment samples; 2) if the presence of PAHs is related to boating activity. Water and sediment samples were collected from sites in the reservoir and local tributaries during a time of high boating activity in June (2034 boats launched at Fountainhead) and during a time of low boating activity in October (653 boats launched at Fountainhead).

METHODS
Samples were obtained from thirty-two sites in the Occoquan Reservoir in June, 1990 and thirty-three sites in October, 1990. Sites were selected to represent areas of both high and low boating activity. Approximately one third of the sites were located near marinas, and the remaining sites were located in non-marina areas throughout the reservoir and tributaries.

For water samples, grab sampling from a motor boat was employed. Water samples were obtained in a Kemmerer sampler, and samples were stored in amber glass bottles. The water samples were extracted according to EPA Method 3510 (in SW-846). This involved liquid-liquid extraction of a one liter sample with methylene chloride, then concentrating the extract to one milliliter in a K-D apparatus. The extract was analyzed by GC/MS (EPA Method 8270 in SW-846).

Grab sampling from a motor boat with an Eckman dredge was employed for soil samples, and samples were stored in amber glass bottles. The soil samples were extracted according to EPA Method 3540. A ten gram sample was soxhlet extracted with 50/50 hexane/acetone, then concentrated and solvent exchanged to hexane only in a K-D. The hexane extract was applied to an alumina column; an aliphatic fraction was eluted with 15 ml of hexane, then the PAH fraction was eluted with 100 ml of methylene chloride. The PAH fraction was concentrated to 1 ml and analyzed by GC/MS (EPA Method 8270).

A Hewlett-Packard 5790 MSD coupled to a Hewlett-Packard 5890 Series II GC was used for analysis. A 30 meter DB-5 column was used, and the temperature program was from 40-320 °C. Full scanning from m/z 50-550 was utilized. A five point calibration curve and single ion quantitation were used for quantitative analysis. Benzo(a)anthracene, anthracene, and chrysene were not resolved under the conditions utilized.

RESULTS
The average concentrations of PAHs found in the water samples for marina and non-marina sites during June, 1990 are listed in Table 1. No PAHs were detected in water samples obtained from the October 1990 sampling trip.

Average concentrations of PAHs in the sediment samples taken from the three different marinas located on the Occoquan Reservoir are listed in Table 2.
TABLE 1: Average Aqueous Concentrations of Marina Sites and Non-Marina Sites during June 1990

<table>
<thead>
<tr>
<th>PAH</th>
<th>Marina</th>
<th>Non-Marina</th>
</tr>
</thead>
<tbody>
<tr>
<td>acenaphthene</td>
<td>(0.21)</td>
<td>ND</td>
</tr>
<tr>
<td>acenaphthylene</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>anthracene</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>benzo(a)pyrene</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>chrysene/benzo(a)anthracene</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>fluoranthrene</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>fluorene</td>
<td>0.19</td>
<td>ND</td>
</tr>
<tr>
<td>naphthalene</td>
<td>0.15</td>
<td>ND</td>
</tr>
<tr>
<td>phenanthrene</td>
<td>(0.37)</td>
<td>ND</td>
</tr>
<tr>
<td>pyrene</td>
<td>0.51</td>
<td>ND</td>
</tr>
</tbody>
</table>

*() slightly below Method Detection Limit
ND = Not Detected

TABLE 2: Average Soil Concentrations at Marinas in June 1990

<table>
<thead>
<tr>
<th>PAH</th>
<th>Bull Run</th>
<th>Fountainhead</th>
<th>Lake Ridge</th>
</tr>
</thead>
<tbody>
<tr>
<td>acenaphthene</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>acenaphthylene</td>
<td>41</td>
<td>22</td>
<td>ND</td>
</tr>
<tr>
<td>anthracene</td>
<td>ND</td>
<td>82</td>
<td>ND</td>
</tr>
<tr>
<td>benzo(a)pyrene</td>
<td>ND</td>
<td>32</td>
<td>22</td>
</tr>
<tr>
<td>chrysene/B(a)A</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>fluoranthene</td>
<td>44</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td>fluorene</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>naphthalene</td>
<td>72</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>phenanthrene</td>
<td>19</td>
<td>39</td>
<td>25</td>
</tr>
<tr>
<td>pyrene</td>
<td>69</td>
<td>27</td>
<td>27</td>
</tr>
</tbody>
</table>

ND = not detected

**DISCUSSION**

Boating activity on the reservoir appears to result in increased petroleum contamination of the reservoir water. During the period of high boating activity in June 1990, sub ug/L quantities of fluorene, naphthalene, and pyrene were detected in the water samples taken from areas where the marinas are located, while no PAHs were detected in corresponding non-marina areas during the same time period. Additionally, samples taken in October, 1990 during a period of low boating activity showed no detectable levels of PAHs in the water. PAHs were detected in the sediment samples taken at the marina sites, further suggesting that boating activity is related to PAH occurrence in the Occoquan Reservoir. To conclusively evaluate the effect of boating on PAHs in the sediment, it would be necessary to compare sediment samples from both marina and non-marina areas.
Determination of Aqueous Cyanide by Purge and Trap Gas Chromatography/Mass Spectrometry

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Preliminary experiments were conducted to ascertain the feasibility of analyzing water and blood samples for cyanide by purge and trap gas chromatography/mass spectrometry.

**Equipment:** A Tekmar LSC-2 purge and trap apparatus was interfaced with a H-P 5890A gas chromatograph, containing a Restek 502.2 volatiles fused silica capillary column. The effluent of the column was fed into a Nermag R10-10H quadrupole mass spectrometer. The mass spectrometer was operated in full-scan mode (m/z=20 to m/z=150).

**Procedure:** Refer to Figure 1. Blood was diluted 1/10 with 0.001 N NaOH, and 4 drops Antifcam-A were added; the solution was shaken vigorously. At this stage, it was treated as per water: 5 mL were added to a gas-tight syringe containing a small stir bar. An internal standard was added and the solution was stirred for one minute. Then bleach and HCl were added to the syringe and the mixture was stirred again one minute. The sample was then placed into a 5-mL sparger, purged for fifteen minutes, and the vapor analyzed for cyanogen chloride by gas chromatography/mass spectrometry. (This method will be referred to as CN-Cl P&T GC/MS.)

**Variation of reaction pH:** Figure 2 shows recovery of cyanogen chloride vs. the pH of the bleach/HCl reaction mixture.

**Variation of bleach concentration:** Figure 3 illustrates recovery of cyanogen chloride vs. the reaction bleach concentration.

**Linearity:** Figure 4 shows the ion chromatogram peak area at m/z=61 ([13CHN]) divided by the ion chromatogram peak area at m/z=62 ([12CHN]) vs. HCN concentration, using [13CHN] as an internal standard.

**Reproducibility:** On seven consecutive days, HCN at 50 ppb (ug/L) was analyzed by CN-Cl P&T GC/MS, using [13CHN] as an internal standard. Figure 5 shows the ion chromatogram peak area at m/z=61 ([13CHN]) divided by the ion chromatogram peak area at m/z=62 ([12CHN]).

**Detection limits:** The full-scan (from m/z=20 to m/z=150) detection limit was approximately 5 ppb HCN. The selected ion monitoring (SIM) detection limit was approximately 0.5 ppb. Both detection limits were based on a signal-to-noise ratio of at least three-to-one for each peak at m/z=61 and at m/z=63.

**Metal complexes:** Cyanide complexes of gold, cobalt, iron, mercury, copper, silver, and zinc were dissolved in 0.001 N NaOH, and analyzed by CN-Cl P&T GC/MS. HCN recoveries were 0 % for complexes of gold and cobalt; 0.14 % for the complex of iron; 83 % for the complex of mercury; 100 % for the complex of copper; 125 % for the complex of silver; and 185 % for the complex of zinc.

**HCN:** The detection limit for purged HCN (no Cl2) was about 500 ppb. Low-level HCN analysis by this method was thus not feasible.

**CN-Br:** The detection limit for HCN was about 300 ppb when CN-Br was generated by HBr and Br2. Low-level HCN analysis by this method was thus not feasible.

**Interferences:** In addition to thiocyanate, methanol when present in the sparger can generate an ion chromatogram peak at m/z=63 at the same retention time as CN-Cl. Vinyl chloride interferes with the use of [13CHN] as an internal standard.

**Blood:** Whole blood was spiked at 500 ppb HCN. Recovery was 99 %.
Recovery of CNCI as a function of reaction pH

Recovery of CNCI (at 100 ppb HCN) vs. bleach concentration

Figure 1

Linearity: Internal Standard + $^{13}$CN

7-day reproducibility (at 50 ppb)

Figure 2

Figure 3

Figure 4

Figure 5
MASS SPECTROMETRIC ANALYSIS OF SAMPLES FROM AN 1864 MILITARY ENCAMPMENT

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Introduction: In September of 1864, the 32nd U.S. Colored Infantry spent about one month at Camp Baird (located on Hilton Head Island, SC, site 38BU79/1151) constructing earthworks for an adjacent fort. As part of the environmental impact study for a proposed golf course and housing development at the site, archaeological excavations were completed near the supposed tent of the regimental surgeon.

Sample A -- Prepared as a diethyl ether extract of tan crystals, and run by direct-insertion probe introduction with El and then with positive ion ammonia and isobutane chemical ionization. The total-ion-current trace showed no strong peaks. A weak peak desorbed at a high temperature gives a mass spectrum with a characteristic isotopic signature at m/z 133, 135, and 137 that matches the envelope predicted for ZnCl2. Zinc chloride can vaporize from the probe at the indicated temperature.

Sample B -- Prepared as a diethyl ether extract of a yellow/green powder, and analyzed by direct-insertion probe introduction with electron ionization. Sulfur vaporized from the direct-insertion probe at a relatively low temperature, consistent with the behavior of pure sulfur. This was confirmed by a search of the spectral library that gave an excellent match to molecular sulfur. Positive ion ammonia and isobutane chemical ionization confirmed the presence of elemental sulfur in this sample. No other significant peaks were noted. GC/MS analysis of an aliquot of the same ether extract (programmed to 250 degrees) showed only a few small peaks that could not be identified.

Sample C -- Prepared as a diethyl ether extract of a black/red chunky solid, and analyzed by direct-insertion probe introduction with El and positive ion ammonia and isobutane Cl. The total-ion-current trace showed no strong peaks with the exception of a peak vaporized at about 240 degrees. This compound searched through the library of electron ionization mass spectra as a phthalate ester, a common plasticizer in plastic bags such as used to store the sample removed from the site. At a temperature of about 400 degrees, higher mass ions in the range of 300 to 500 amu were observed. Reconstructed ion chromatograms show the reproducible desorption of this ion series at higher probe temperatures. Interestingly, these ions provide a good library match to safflower oil. While the identification is not confirmed as that particular oil, the spectral signature is consistent with that of a naturally derived plant oil. Neither ammonia nor isobutane chemical ionization mass spectrometry (using the same temperature program from the probe) could provide supporting evidence for the presence of this natural oil. Positive ion fast atom bombardment (FAB) mass spectra in a meta-nitrobenzyl alcohol matrix does give some high mass peaks separate from matrix background, suggesting the presence of high mass organic acids, but the spectrum could not be uniquely identified.

Sample D -- This sample was prepared as a diethyl ether extract of a brown amorphous mass, and analyzed by direct-insertion probe El and positive ion ammonia and isobutane Cl. The total-ion-current trace with electron ionization showed several moderately strong peaks for compounds that vaporized from the probe at about 225 and 400 degrees. The first peak produced an electron ionization mass spectrum that matched the library search as octadecanoic acid. The second peak produced an electron ionization mass spectrum that matched as an ester of octadecenoic acid. The presence of the higher mass ions in the latter mass spectrum is significant. Although the match to any one of the compounds shown in the library is not good, the similarity in compound class for all of the compounds listed shows that a mixture of related compounds is vaporizing at the same time from the direct desorption probe. These compounds are characteristic of extracts from a number of plant products, the exact nature of the preparation cannot be determined. Both ammonia and isobutane positive ion chemical ionization produce total ion current traces similar to that measured for electron ionization, and evidence for the same classes of compounds vaporizing from the probe. Positive ion FAB mass spectra (meta-nitrobenzyl alcohol matrix) confirms the presence of such high mass compounds.

Sample E -- Prepared as a diethyl ether extract of a dark brown amorphous mass still in the original ceramic container, and analyzed by direct-insertion probe El. Several peaks were observed.
in the trace of total ion current as samples evaporated from the probe. Again, these compounds provide electron ionization mass spectra that are matched by the computer library with C14, C16, and C18 acids and their derivatives. Positive ion isobutane chemical ionization mass spectra confirm the mass spectra and the classes of compounds observed. A close similarity in the desorption profile and the mass spectra to those observed for Sample D is observed.

Sample F – This sample is a bottle filled with a clear liquid with a flocculated precipitant. The condition of the stopper suggests that the seal was intact. Several samples were derived, including a diethyl ether extract of the supernatant (F1), an ether extract of the precipitate (F2), and an ether extract from the cap itself (F3). EI mass spectra were recorded from a temperature-programmed direct insertion probe run for each. GC/MS analyses were similarly completed; in one case, a silylation was performed using BFTBA to increase the volatility of any components present and allow their passage through the GC column. Finally, FAB analyses with both dithiothreitol/dithioerythritol and m-nitrobenzyl alcohol liquid solvents were completed.

For F1, the probe electron ionization run shows only a small peak vaporizing at a temperature of about 400 degrees. Since most of the peaks in the mass spectrum are also present in the mass spectrum of a compound that vaporizes much earlier, and identified as a plasticizer, the identity of the latter peak cannot be established. An ion at m/z 149 normally indicates the presence of a phthalate plasticizer. However, an analysis of the ether blank was performed separately to confirm that we have not introduced such a contaminant in sample preparation. For F2, the EI mass spectra from the precipitant are very similar to those recorded from the liquid solution, as are the electron ionization mass spectra recorded for sample F3. Sample F1 was silanized and the GC/MS analysis completed. Library search of the EI mass spectra give indication for silylated hydrocarbons derived from the column packing. Significantly, the trimethylsilyl derivative of arsenous acid is indicated.

Discussion: The results obtained from the mass spectrometric analyses must be considered in view of the drugs likely to be present in the samples. Robertson and Damann have authored monographs that describe the pharmacopoeia of the times. The list of tinctures, extracts, and derivatives is extensive, and includes quinine, ethers, chloroform, morphine derivatives, and opium preparations (opium in one form or the other was used to treat almost every infirmity or condition).

Zinc chloride, zinc acetate and zinc sulfate are all listed as standard issue for physicians. Sample A was identified as zinc chloride (butter of zinc). This compound was usually issued as a solution, and was used as a disinfectant, a deodorant, and embalming preservative. Similar uses are noted for zinc acetate and sulfate. The original status of the sample at the site (whether in a bottle or not) was not noted. Sample B was determined to contain sulfur. Sulfur was issued to physicians by the pound; it was used as a scabicide (often mixed with lime), an antiseptic, and in ointments used to treat parasites. Sample E was thought to be the ubiquitous blue mass when first submitted to the laboratory. Blue mass contains 32-34% elemental mercury, along with powdered licorice, powdered rose leaves, and honey. Blue mass and calomel were widely used cathartics, diuretics, and antiseptics. They were consumed internally and applied externally. Systemic mercury poisoning often resulted; however, such was the medicine of the time, and it is instructive to note that mercury ointments were used as topical treatments for syphilis as late as the 1940s. However, examination by FAB, EI, or CI did not provide any evidence for the presence of mercury in this sample. We cored the sample to test whether mercury was still present in the unexposed core of the sample; this sample did not show the presence of mercury. The same sort of porcelain apothecary jar usually used to hold blue mass was also widely used to contain cerates, a mixture of bland fats and oils used as a salve to promote healing. Without a standard composition for such a sample, we can only suggest that this is the composition. In any case, the initial characterization of the sample as blue mass was incorrect. Sample F provided spectral evidence for the presence of arsenous acid, provided at the time to be used in the cauterization of wounds. For samples C and D, the presence of any particular compound could not be unequivocally established. Safflower oil was listed as a possible compound for sample C, and long chain fatty acids for sample D. We have insufficient experience to identify these samples further. Physicians of the time were equipped with extracts of a large number of plants. Without standards, more accurate determinations could not be made.
Results of experiments in our laboratory have shown that benzene is metabolized by animals in part to an intermediate which binds to cysteine groups in hemoglobin to form the adduct S-phenylcysteine (SPC). These results suggested that SPC in hemoglobin may be an effective biological marker for exposure to benzene. However, we could not detect SPC in the globin of humans occupationally exposed to benzene concentrations as high as 28 ppm for 8 h/day, 5 days/wk. As an alternative biomarker for humans, we examined the binding of benzene to cysteine groups of a different blood protein, albumin. To facilitate the process, a new method for the precipitative isolation of albumin from plasma was developed (Fig. 1). The isolated albumin was analyzed for SPC by isotope dilution gas chromatography/mass spectroscopy. We used this approach to measure SPC in the albumin of F344/N rats exposed by gavage to 0 - 10,000 μmoles/kg benzene. Amounts of albumin-associated SPC increased rapidly as a function of dose, followed by a leveling off in the amount of SPC seen at higher doses. Levels of SPC were measured in humans occupationally exposed to average concentrations of 0, 4.4, 8.4, and 23.1 ppm benzene 8 hr/day, 5 day/wk. Of nine controls, seven had levels of SPC below the limit of detection (0.1 pm SPC/mg albumin). SPC-albumin adducts increased in the blood of exposed groups in a linear fashion relative to exposure concentration, although only the highest dose group possessed levels statistically different from controls (Fig. 2). Current research focuses on the occurrence and extent of SPC in individuals with no known exposure to benzene, and in cigarette smokers, since exposure to cigarette smoke is the most common mode of benzene exposure in the United States. SPC in albumin may prove to be useful biomarker of benzene exposure. (Research sponsored by the U.S. DOE/OHER under Contract No. DE-AC04-76EV01013.)
ISOLATION OF BLOOD PROTEIN

WHOLE BLOOD

SPIN

RBC

PLASMA

0.5 M CaCl₂

SUPERNATANT

FIBRINOGEN

ACID/ALCOHOL

SUPERNATANT

GLOBULINS

NaOAc/ETOH

SUPERNATANT

ALBUMIN (>95% PURE)

Figure 1. Scheme for isolation of albumin from whole blood

Figure 2. S-phenylcysteine in albumin of Chinese workers exposed by inhalation to 0, 4, 8, 4 and 23.1 ppm benzene over 8 h/day, 5 days/wk. Results are means ± standard errors. There were nine, four, three, and five workers in each group, respectively.
ARSENIC METABOLITE ANALYSIS BY MASS SPECTROMETRY

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Introduction. Arsenic is found widely distributed in the atmosphere and on the earth's crust as well as in the aquatic environment. The mechanisms of arsenic toxicity are related to the oxidation state of the compound. It is generally accepted that trivalent arsenic (As(III)) exerts its toxic effects by reacting with thiol groups which are often important parts of active centers for many enzymes. Pentavalent arsenic (As(V)) can substitute for inorganic phosphate due to a similarity in structure. Apparently two major mechanisms are involved in the detoxification of inorganic arsenic by mammals. These involve methylation and protein binding (1). Biomethylation appears to be the predominant path for detoxification of inorganic arsenic in both mammals and other organisms. Biomethylation of inorganic arsenic by mammals leads to the production of methylarsenic acid (MA) and dimethylarsenic acid (DMA) which are mainly excreted in the urine. A need exists to be able to separate out the various arsenic species due to their different toxicities. At the present time, separation by ion-exchange HPLC and quantification by either atomic emission or absorption spectrometry appears to show the most promise. The use of HPLC procedures allows the separation and purification of most compounds from a complex mixture. Analysis by mass spectrometry can provide immediate structural and molecular weight information. The objectives of the present study were to: (a) develop a simple, reproducible method for the structural analysis of reduced metabolites of alkylarsines utilizing mass spectrometry (MS), and (b) develop procedures to minimize possible molecular rearrangement of alkylarsines generated from aqueous mixtures of organic arsines.

Materials and methods. Highest grades available of sodium arsenite (NaAsO₂, As(III)), sodium arsenate (Na₂H₂AsO₄·7H₂O, As(V)), sodium methylarsonate (CH₃NaH₂AsO₄) and dimethylarsinate ((CH₃)₂NaAsO₂) were used. Stock solutions of the arsenic standards were prepared weekly in deionized water (1 mg/ml) and kept refrigerated until use. Sodium borohydride (NaBH₄, 98%) was purchased from Mallinckrodt. Working standards were diluted from stock solutions with deionized water and prepared on the day of analysis.

Animals and liver cytosol preparation. Male Wistar rats (250-300g) were sacrificed by decapitation and livers were immediately removed and rinsed in ice cold 0.32M sucrose in 50mM HEPES buffer pH 7.40, blotted dry on filter paper and weighed. A 50% homogenate was then prepared (40g/80ml buffer) in the above buffer using a Potter-Elvenhjelm homogenizer (Teflon pestle). The cytosol was then prepared by ultracentrifugation of the homogenate at 100,000 x g for 75 min at 4°C in a Beckman Model L3-40 ultracentrifuge (50.2TI rotor). The supernatant was then pipetted off and kept on ice for preparation of the standard incubation mixture (SIM).

Preparation of SIM. The SIM was prepared with 1 ml of 0.32M sucrose in 50mM HEPES buffer, pH 7.4, 0.75 ml of a methyl-cobalamin solution (10mM final concentration in SIM), 0.25 ml of 10mM As(V), and 0.5 ml of cytosol. The mixture was incubated for 60 min in a water bath at 37°C in open glass tubes. The reaction was stopped by the addition of 0.5 ml 15%(w/v) trichloroacetic acid. The supernatant was then pipetted off and kept on ice for preparation of the standard incubation mixture (SIM).

Hydride generation and analysis of arsines. As(III) and DMA were reduced with 5% NaBH₄ in 0.1N NaOH in a pH 4.0 solution following deoxygenation with N₂ gas. Aqueous samples of As(V) and MA were analyzed by first acidifying the samples in 3M H₂SO₄ followed by reduction with NaBH₄ as described by Lakso et al. (2). The volatile arsines were collected in a glass expansion chamber attached to an arsine generator. The expansion chamber was then disconnected from the generator and connected to a glass "U"-trap. The arsine gas was then slowly bled into the liquid nitrogen cooled trap that was being pumped out under vacuum and trapped. The cryogenic trap was then removed and the arsines volatilized at their boiling points and were pumped by vacuum into the MS for analysis. The instrument used was a UTI Model 100B quadrupole mass spectrometer that was part of a Varian CVA-1 vapor analysis apparatus. The mass spectrometer
was run at an electron energy of 70eV. Mass spectra were recorded with a Gould Brush 220 recorder system.

**Cation-exchange chromatography.** Arsenic metabolites in the SIM were first separated by cation-exchange chromatography according to the method of Tarn et al. (3).

**Results.** Reaction rates for arsine generation were monitored qualitatively with the use of mercuric chloride-saturated filter paper disks (Gutzeit test for arsine). Peak arsine formation was seen at 45 sec from the start of NaBH₄ addition. When As(III) and As(V) were reduced with 5% NaBH₄, the spectra showed peaks at 75, 76, 77 and 78 m/z corresponding to arsine (AsH₃). With MA, reduction peaks were observed at 75, 76, 77, 78, 90 and 92 m/z indicating masses for arsine and methylarsine (CH₃AsH₂). When DMA was reduced with 5% NaBH₄, masses were observed for arsine and methyarsine. However, when 3% NaBH₄ was used, in addition to those masses observed above, masses at 105 and 106 were seen indicating dimethylarsine. Under our experimental conditions, mass spectra generated from As(V) and MA were observed only at pH 1 but not at pH 4. Mass spectra generated from DMA occurred only at pH 4 but not at pH 1 but spectra from As(III) were observed at both pH 1 and pH 4. The approximate lower limits of detection for MA and DMA was 500ng and 1ug for As(III) and As(V). Preliminary in-vitro biomethylation experiments utilizing separation by cation-exchange chromatography prior to hydride generation (5% NaBH₄) showed spectra for arsine and methyarsine at pH 1 and 4 in fractions that did not elute for MA and DMA.

**Discussion.** Although AsH₃ from As(III) could be generated from both pH 1 and 4, AsH₃ from As(V) was only found when the reduction occurred at pH 1, not at 4. MA and DMA generated arsine and methyl- and dimethylarsine at pH 1 and 4 respectively, supporting the suggestion by Aggett and Aspell (4) that arsines can be selectively generated from a complex mixture by changing the pH. Procedures such as the use of glass beads or silanized glass wool that are used in the "U"-trap to increase the recovery when measuring arsine gases with atomic absorption spectrophotometry or a DC discharge cell (5,6,7) were not effective in our system which uses MS for detection. We were unable to observe any spectra with the use of our "U"-trap half-packed with glass beads. Furthermore, using 0.4% silanized glass wool in the trap resulted in the absence of spectra for arsine and the spectra were greatly suppressed for methyarsine. The design of our system does not utilize any trapping material in the "U"-tube and places a fiber plug between the generator and the gas expansion chamber to break-up aerosol and thus decreased the amount of water vapor which decreased the suppression of the arsine mass spectra. The lack of dimethylarsine peaks between 100-110 m/z with 5% NaBH₄ may be explained by possible arsine molecular rearrangements as noted by others (8). This observation appears to be dependent on the initial NaBH₄ concentration because when a 3% solution was used we were able to detect the spectra for dimethylarsine. The results of the in-vitro biomethylation experiments using rat liver cytosolic fractions and As(V) showed mass spectra for methyarsine with 5% NaBH₄. These fractions did not contain MA or DMA, thus suggesting that the methyarsine detected by MS was generated from some other unknown methylated arsenic metabolite. This would also suggest the possibility that the methyl- and dimethylarsine observed in other studies not utilizing chromatographic techniques prior to hydride generation could result in other interpretations of their data. Further work is necessary in order to unequivocally identify our unknown methylated arsenic metabolite.

**References.**

Characterization of Naphthalene Metabolite-Peptide Adducts using Mass Spectrometry

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Exposure to naphthalene is a potential health hazard for cigarette smokers and industrial workers. Naphthalene exerts species and organ specific toxicity that is not yet fully understood. The goal of this study is to help us understand naphthalene's potential toxicity to humans, and the toxicity of polyaromatic hydrocarbons in general. The metabolites of naphthalene used in these experiments can serve as simple models that will help us learn more about the reactivity of electrophilic metabolites of polyaromatic hydrocarbons toward nucleophilic macromolecules such as proteins. In addition to studying the reactivity, the characterization of the products formed will provide important information towards understanding the mechanism of toxicity that polyaromatic hydrocarbons undergo.

Two of the reactive metabolites studied here are aromatic quinones. Therapeutic drugs and some pesticides contain quinones, but quinones are widely distributed in nature and are found in higher plants, fungi, bacteria, and throughout the animal kingdom as well. Quinones can exhibit toxicity by forming covalent adducts or by redox cycling. The two quinones included in these experiments may also serve as models that will further the knowledge of how this important class of compounds reacts towards protein nucleophiles.

The present work has investigated the reactions of three metabolites of naphthalene (1,2-naphthoquinone, 1,4-naphthoquinone, and racemic 1,2-naphthalene oxide) with model compounds N-acetylcysteine, α-N-acetyllysine, Lys-Tyr-Lys, and Lys-Cys-Thr-Cys-Cys-Ala. Aqueous solutions (pH = 7.4) of these reactants were incubated for 24 hours on a shaking water bath at 37°C. The mixtures were then lyophilized.

The crude reaction mixtures, and HPLC fractions of the adducts formed from incubations of the naphthalene metabolites with model peptides, were analyzed by static FAB in positive ion mode. In all instances, the sulfhydryl (cysteinyl) and amino (lysinyl) groups reacted in a similar fashion resulting in Michael addition products that are further oxidized to yield the quinone adduct (rather than the hydroquinone adduct). Schiff base adducts are only seen in minor amounts. Especially interesting were the spectra obtained from the adducted peptides.

In the case of the mono-adduct tri peptide of Lys-Tyr-Lys, tandem mass spectrometry was performed on the parent ion which consisted of the Lys\textsubscript{1}-adduct and the Lys\textsubscript{3}-adduct. Figure 1 shows the daughter ion spectrum obtained by collisionally activated dissociation of the [M+H]\textsuperscript{+} m/z = 595, with the especially relevant daughter ion peaks annotated.

The spectrum provided conclusive evidence that the quinone is binding to either the alpha position on the N-terminal lysine or at the epsilon position of the C-terminal lysine.

Incubation of Lys-Cys-Thr-Cys-Cys-Ala with naphthoquinone produced a mixture of products ranging from the mono-adduct through to the tetra-adduct and these are evident in the spectrum shown in figure 2. In addition to forming multiple adducts, the products are in various oxidation states as shown by the multiplicity of the peaks. The adducts detected here may be in the quinone or the hydroquinone form, or a combination of the two where more than one modification has occurred. The excess unreacted quinone oxidized the sulfhydryl groups on the cysteine to form a disulfide linkage.
The results indicate that the quinones form Michael adducts even with amino groups. Sequence information that indicates the position of the adduct within the peptide is obtained from daughter ion spectra. In addition, the bound quinone may be inducing oxidation to occur elsewhere within the peptide.

[Figure 1: Daughter ion spectrum of Lys-Tyr-Lys/Naphthoquinone adducts, MW = 382]

[Figure 2: Spectrum of Lys-Cys-Thr-Cys-Cys-Ala/Naphthoquinone adducts]

Acknowledgement
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References
Leukotrienes and hydroxyeicosatetraenoic acids (HETEs) are metabolites of arachidonic acid. The more common members of the leukotriene family of compounds are formed in vivo by a cascade of reactions initiated by a lipooxygenase-catalyzed oxygenation of arachidonic acid. All six possible hydroperoxide (HPETEs) that could result from such a reaction are formed in one cell type or another. After the initial oxygenation, the HPETEs can undergo several different transformations. The HPETEs are often reduced to hydroxy derivative (HETEs) or are dehydrated to the initial members (LTA4s) of the leukotriene family. Subsequent hydrolysis to LTB4 or reaction with glutathione leads to the other leukotrienes. Many of these various derivatives are known to be involved in a multitude of physiological processes, including hypersensitivity reactions and inflammation. As part of our continuing interest in the FAB/MS/MS analysis of biomolecules, we have investigated the collisional activation decomposition of these compounds using coaxial continuous flow FAB (1,2).

Coaxial continuous flow FAB has proven to be a useful technique for the acquisition of MS/MS spectra (3,4). The coaxial CF-FAB interface uses a pair of coaxial fused silica capillary columns to independently deliver the column analytes and the FAB matrix to the FAB probe tip (1,2). This design is advantageous since the chromatography is not affected by the matrix and one gains independent optimization of the composition and flow rates of the analytes and the FAB matrix. In addition, the coaxial CF-FAB interface allows for increased sensitivity over other CF-FAB Interfaces due to the ability of the a nanoscale capillary LC system to deliver a high concentration of analyte in a short time period. This advantage makes this interface very useful for the acquisition of MS/MS spectra. The MS/MS spectra of a variety of compounds have been acquired by delivering a constant flux of analyte into the mass spectrometer (3,4). This constant flux of analyte is advantageous for the acquisition of CAD spectra that are otherwise cumbersome. We demonstrate the use of this constant flux of analyte with the acquisition of the MS/MS spectra of a variety of leukotrienes and HETEs.

The leukotrienes and HETEs were desorbed by FAB as negative ions and then collisionally activated. CAD spectra of the (M - H)- ions of these derivatives of arachidonic acid reveal that structurally informative fragmentations occur. These fragment ions can be used to locate the position of hydroxy groups on the fatty acid chain. In addition, the position and type of the cysteine moiety of the leukotrienes can be determined. As an example, the MS/MS spectrum of LTC4 is shown in Figure 1. The fragmentations observed for high energy collisions are different than those observed by Gaskell and coworkers (5) for low energy collisions. Upon low energy collisional activation, LTC4 fragments to give an abundant ion of m/z 306. Increased fragmentations involving extensive H-transfers around the S of the cysteine moiety are observed at high collision energies. For example, the LTC4 fragment ions of m/z 306 observed in low energy collisions shifts to m/z 305 at high collision energies.

Upon collisional activation, the HETEs fragment to give an abundant loss of water. The MS/MS spectrum of the (M - H)- ion of 12(S)-HETE is shown in Figure 2. In addition, fragmentations on either side of the substituted carbon are particularly facile. These fragmentations are especially useful for the determination of the location of the hydroxy substituents.
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References:

Figure 1

Leukotriene C4
MS/MS of m/z 624

Figure 2

Leukotriene B4

748
The epoxyeicosatrienoic acids (EETs) are biologically active eicosanoids derived from arachidonic acid and a cytochrome P450 mono-oxygenase (1). Insertion of an oxygen atom into one of the four double bonds in arachidonic acid produces four regioisomers of EET: 5,6-, 8,9-, 11,12-, or 14,15-EET. Detection of EETs in biological systems is difficult mainly because of the rapid metabolism of these compounds to dihydroxy fatty acids, glutathione conjugates, or reincorporation into cellular lipids. The strongest evidence that the EETs are endogenously formed is the discovery of 14,15- and 8,9-EETs in urine (2). In kidney and liver as much as 80% of the EETs were found incorporated into phospholipids (3–5). In order to investigate the physiological significance of the EETs or the EET-phospholipids, adequate analytical methods are required for direct structural characterization of these labile molecules.

Analysis of EETs has mostly been done by GC/MS of the free EET after phospholipase A2 treatment of the phospholipids (4). In this work we show that fast atom bombardment ionization and tandem mass spectrometry can be used to directly analyze the different isomers of EET incorporated into phospholipids from biological sources (6).

### METHODS

**Incorporation of EETs into mast cells** - Virally-transformed murine bone marrow-derived mast cells kept in tissue culture were harvested (1.6–2.6 × 10^8 cells) by centrifugation at 50×g for 15 min at room temperature, washed then resuspended in Hank’s balanced salt solution to 0.7 × 10^7 cells/ml. Individual EETs were dissolved in 5mg/ml fatty acid free bovine serum albumin (BSA) and then added to cells to give final concentrations of 1μM EET and 0.05mg/ml BSA. Incubations proceeded at 37 °C for 2 hours. Incubations were stopped by centrifugation and washing the cells to remove any unincorporated EET. EETs (5,6-, 8,9-, 11,12-, and 14,15-EET) were obtained from Cayman Chemical, Ann Arbor, MI.

**Isolation** - Phospholipids were extracted from the cells using CH₃0H/CHC1₃/H₂O (1:1:1) according to the method of Bligh and Dyer (7). The phospholipids were then separated according to polar head groups by normal phase HPLC, using a Lichrosorb column (4.6x 250mm, 3μm, Phenomenex, Torrence, CA). The mobile phase consisted of a gradient of 53% solvent A(hexane/isopropanol; 30/40; v/v) held for 6 min then programmed to 100% solvent B (hexane/isopropanol/H₂O; 30/40/7; v/v/v) over 20 minutes. Individual molecular species within each phospholipid class were separated using reverse phase HPLC with an Ultrasphere ODS column (4.6x 250mm, 5 μm; Beckman, San Ramon, CA). Mobile phase consisted of CH₃OH/H₂O/CH₃CN (90:5.7/2.5; v/v/v) containing 1mM NH₄OOCFCF₃.

**Mass Spectrometry** - Collisionally induced dissociation mass spectra of isolated EET-phospholipids and authentic unesterified EETs were obtained from 20–100 ng of material, using negative FAB ionization and a triple quadrupole mass spectrometer (Finnigan TSQ70B, San Jose, CA). Diethanolamine was used as the FAB liquid matrix. The fast atom gun was operated at 1mA with xenon accelerated to 6 kV. Argon (0.5 mtorr) was the collision gas. The collision offset energy (E₀ab) was 30 eV. The electron multiplier conversion dynode was maintained at 12 kV.

### RESULTS

**Negative ion FAB** collisionally induced dissociation mass spectra of the [M-H]⁻ ion from 16:1p/EET GPE (1-O-hexadec-1'-enyl, 2-O-epoxyeicosatrienoyl-glycerophosphoethanolamine) molecular species isolated from mast cells that were incubated with either 14,15-EET, 11,12-EET, 8,9-EET, or 5,6-EET as well as the negative FAB CID mass spectra of the [M-H]⁻ ion from unesterified 14,15-, 11,12-, and 8,9- and 5,6-EET are shown in Table 1. Different low mass fragment ions were observed in the CID mass spectra of each of the four 16:1p/EET GPE molecular species. CID mass spectra of the four isomeric EETs, in unesterified form, were taken to test the origin of these low mass ions. The same low mass fragments that were observed upon CID of the EET-containing phospholipid molecular species were observed in the CID spectra of the corresponding unesterified EETs. Possible mechanisms for some representative fragmentations are shown in figure 1.

### CONCLUSIONS

Collisionally induced dissociation of EET-containing phospholipid molecular species yields low mass ions that are indicative of the specific regioisomer of the esterified EET substituent. Negative FAB and CID are useful for...
identifying EET-containing phospholipid molecular species isolated from biological sources at the 100 ng level. The EET-specific ions from EET-phospholipids probably arise from secondary fragmentations of the EET carboxylate anion (m/z 319) that is produced upon CID of the phospholipid [M-H]⁺ ion. Some collisionally induced dissociations of the EET ion appear to arise by charge-driven mechanisms that may involve the transfer of allylic or bis-allylic protons to the carboxyl moiety leaving an anionic site on the hydrocarbon chain.

**Acknowledgement** - This work was supported in part by a grant from the National Institutes of Health (GM41026).

**REFERENCES**


**TABLE 1.** Series of fragment ions produced by CID of [M-H]⁺ of 16:1p/EET-GPE and unesterified EETs.

<table>
<thead>
<tr>
<th>isomer of EET</th>
<th>Fragmentation m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>[M-H]⁻⁻⁻⁻</td>
<td>RCO⁻⁻⁻⁻ RCOOH⁻⁻⁻⁻ RCOO⁻⁻⁻⁻ RCOO⁻⁻⁻⁻ H₂O⁻⁻⁻⁻ H₂O⁻⁻⁻⁻ CO₂⁻⁻⁻⁻</td>
</tr>
<tr>
<td>14,15- (GPE)</td>
<td>738 436 418 319 301 257 219 207 203 175, 139, 113</td>
</tr>
<tr>
<td>11,12- (GPE)</td>
<td>738 436 418 319 301 257 179 167 163 208</td>
</tr>
<tr>
<td>8,9- (GPE)</td>
<td>738 436 418 319 301 257 139 127 123 203, 151, 155</td>
</tr>
<tr>
<td>5,6- (GPE)</td>
<td>738 436 418 319 301 257 99 87 83 115 163, 191, 115</td>
</tr>
</tbody>
</table>

**Figure 1.** Possible mechanisms for the fragmentation of 11,12-EET resulting in the formation of the (a) "B" ion, by a charge-driven mechanism, and the (b) "C" ion, by a charge-remote mechanism.
ANALYSIS BY FOUR SECTOR TANDEM MASS SPECTROMETRY OF COMPLEX MIXTURES OF PHOSPHATIDYLCHOLINES

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Glycero-type phospholipids, along with protein, are the main constituents of biomembranes. The most abundant glycero-type phospholipid in mammalian cell membranes is phosphatidylcholine which can account for up to 49.5% of the total phospholipid content and up to 30% of the total lipid content in cells. Acyl chain length can vary from 10 to 24 carbons and up to 6 unsaturations per acyl group have been reported.

Fast atom bombardment (FAB) ionization has proven to be the optimal technique for mass spectral analysis of choline-containing phospholipids (1-4). Using a JEOL HX110/HX110 four sector tandem mass spectrometer we have examined the phosphatidylcholine content of a purified preparation of the human immunodeficiency virus (HIV-1), the etiologic agent of AIDS. In the positive ion mode, molecular ion stability is enhanced by the presence of the tetraalkylated ammonium cation, while in the negative ion mode formation of a phosphoryl anion contributes to the stability of the negatively charged fragments. Figure 1 shows the negative-ion FABMS spectrum of the phosphatidylcholine content of the HIV-1/MN virus. When combined with tandem mass spectrometry, not only can complex mixtures be analyzed, but much structural information can be obtained. Positive ion FABMS/MS can be used to identify the polar head group. Negative ion FABMS/MS is useful in elucidating the structure and position of the acyl groups. Figure 2 shows the negative-ion FABMS/MS spectrum of the ions weighing 742 from the viral phosphatidylcholine mixture; it can be seen that two phosphatidylcholines occur at this mass in the mixture. The four sector instrument used here provides high energy collisions, unit resolution in both MS-1 and MS-2, while maintaining good ion transmission and provides the capability to perform MS/MS/MS experiments (5) (Figure 3). The higher resolution of mass selection for a four sector mass spectrometer compared to a two sector instrument is essential for studying phospholipids, many of which differ by only two mass units.

An approximate quantitation of the fatty acid content of the phosphatidylcholines present in the HIV-1 viral preparation was obtained by measuring the relative peak heights of the phosphatidylcholine protonated molecules in FABMS AND FABMS/MS. An array of fatty acids is revealed that is consistent with that found in human blood cells. This work has been submitted to Analytical Chemistry and is in press.

References

Figure 1.
Negative ion FAB/MS spectrum of phosphatidyl cholines from HIV-1/MN virus.

Figure 2. Negative-ion FABMS/MS of l-palmitoyl-2-octadecadienoylglycerophosphocholine and 1-hexadecenoyl-2-octadecenoylglycerophosphocholine.

Figure 3. Negative-ion FABMS/MS/MS of the eicos-11-enoate anion from 1-eicos-11-enoyl-2-hexadecenoylglycerophosphocholine.
Charge Remote Fragmentation during FAB-CAD-MS/MS of Triphenylphosphonium Derivatives of Carboxylic Acids.

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Recently, a simple chemical method has been developed in this laboratory, which permits the triphenylphosphonium (TPP) moiety to be selectively attached to the C-terminus of peptides, thus localizing a positive charge at that site (1). EDC [ethyl-3-(3-dimethylaminopropyl)-carbodiimide] activates the carboxylic acid group, allowing it to be coupled to the amino group of the AETPP (aminoethyl-TPP) reagent. The derivative significantly enhances the efficiency of FAB ionization by increasing the surface activity of the analyte and controls the fragmentation pattern by a remote charge fragmentation process (2). This method has now been extended to various fatty acids.

![Chemical structure](https://example.com/structure.png)

FAB-CAD-MS/MS of these fixed-charge AETPP derivatives of carboxylic acids show typical charge remote fragmentation as shown with metal ion adducts by Gross et al. (3). The fragmentations observed involve the parallel losses of C\(_n\)H\(_{2n+2}\) elements beginning at the alkyl terminus (Figure 1). The AETPP derivatives of tested fatty acids produce diagnostic fragments for locating a variety of structural modifications. For example, methyl branch positions in 4,8,12-trimethyloctadecanoic acid are easily recognized from the FAB-CAD-B/E linked scan spectrum of the M\(^+\) ion of the AETPP derivative (Figure 2). Iso and anteiso branched fatty acids also are easily distinguished by this characteristic fragmentation pattern (Figures 3 & 4).

In comparison to the metal cationized fatty acids, the detection limits for the analytes can be reduced substantially upon preparation of the AETPP derivative. This could be due in part to the fact that the AETPP ions are shifted up in mass by 288 u into a region where the chemical noise is lower and the ion current is concentrated principally in fixed-charge molecular ions of AETPP derivatives. Moreover, the covalently-bonded charged moiety generally allows better control of fragmentation than does the adduct with a metallic cation. The application of this procedure to other acids, including prostaglandins and thromboxanes with comparison of their mechanism of fragmentation to that of metallic cation adducts are currently under investigation.

![Figure 1](https://example.com/figure1.png)
Figure 2. Trimethyloctadecanolc acid-AETPP, FAB-CAD-B/E.

Figure 3. Isopalmitic acid-AETPP, FAB-CAD-B/E.

Figure 4. Antoisomargaric acid-AETPP, FAB-CAD-B/E.

ANALYSIS OF STEROLS IN FUNGI BY TANDEM MASS SPECTROMETRY

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Most fungi have been found to produce sterols in a variety of forms [1]. Tabulation of the content and distribution of the individual sterols present in different fungi may constitute a useful method for fungi identification. Tandem mass spectrometry has the potential to identify various types of sterols in complex mixtures without the use of derivitization.

Samples of crude lipid extracts were introduced directly into the ion source of a triple quadrupole mass spectrometer via a direct exposure probe. Three structural features were studied: $\Delta^5$, $\Delta^{5,22}$, and $\Delta^{5,7}$. Characteristic daughter ions were found for these specific structural features using four sterol standards as examples. The $\Delta^5$ sterols (cholesterol, sitosterol) produced two characteristic daughter ions of m/z 231, and m/z 178, the $\Delta^{5,22}$ sterol (stigmasterol) produced two characteristic daughter ions of m/z 273, and m/z 271, and the $\Delta^{5,7}$ sterol (ergosterol) produced one characteristic daughter ion of m/z 253. The daughter spectrum of the cholesterol molecular ion is shown in Figure 1.

Feature-selective parent scans were then developed for these characteristic fragmentations, and their usefulness confirmed using the sterol standards. Examples of two of these scans are shown in Figure 2. In the first example, a parent scan of m/z 231 for cholesterol, a $\Delta^5$ sterol, produced a peak at m/z 386, which represents the molecular ion of cholesterol. In the second example, a parent scan of m/z 271 for stigmasterol, a $\Delta^{5,22}$ sterol, produced a peak at m/z 412, which represents its molecular ion. Similar results were obtained for ergosterol and sitosterol.

Next, these feature selective parent scans were applied to the crude lipid extracts of four fungi samples. A number of peaks were detected in each sample that represented the molecular ions of different sterols containing the specific structural features for which the samples were being analyzed. Examples of these scans are shown in Figure 3. Variations in these distributions of sterols may provide a basis for detecting, characterizing, and identifying fungi.

The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Cholesterol

Daughters of m/z 386

![Figure 1. Daughter spectrum of the molecular ion of cholesterol, a Δ⁵ sterol. Daughter peaks occur at m/z 178 and m/z 231 that are characteristic of Δ⁵ sterols.]

Cholesterol, Parents of m/z 231

Stigmasterol, Parents of m/z 271

![Figure 2. Feature-selective parent scans for Δ⁵ and Δ⁵,²² structural features. On the left, a parent scan of m/z 231, specific for the Δ⁵ feature, detected the molecular ion of cholesterol. On the right, a parent scan of m/z 271, specific for Δ⁵,²² feature, detected the molecular ion of stigmasterol.]

Figure 3. Examples of the application of feature-selective parent scans to Zygorynchus moelleri. Some of the sterols represented by the spectral peaks have been tentatively identified.
ANALYSIS OF SULFATED GLYCOSAMINOGLYCANS FOR THE DETERMINATION OF SULFATE POSITION USING FAST ATOM BOMBARDMENT IONIZATION AND TANDEM MASS SPECTROMETRY

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Glycosaminoglycans (GAGs) are sulfated, linear polysaccharides found in mammalian tissues. These compounds exhibit a variety of biological properties and can be altered in disease states [2]. The presence of a certain type of GAG or the identification of one with a particular disaccharide composition might give valuable information about the nature of the biological process in which the GAG is involved. To study the structure of GAGs we rely on polysaccharide lyases to break them down into smaller oligosaccharides.

Recent work in our laboratory, with the underivatized sodium salts of sulfated oligosaccharides obtained from heparin, demonstrated that negative ion fast atom bombardment (FAB) mass spectrometry analysis, using the matrix triethanolamine, is useful for both molecular weight and sequence determination [3]. In addition to the loss of NaSO$_3^{-}$ ($+H^+$) ions, first reported by Reinhold and Carr using the matrix triglycerol [4], there was glycosidic cleavage which gave the corresponding sugar sequence. These full scan spectra can be used to determine which sugar ring contains sulfate groups but can not specifically pinpoint the sulfate substitution on the ring.

Our current work focuses on the sulfated disaccharides derived from chondroitin/dermatan sulfate polysaccharides. Exhaustive treatment of these chondroitin sulfates with chondroitin AC and ABC lyases [5] affords a mixture of unsaturated disaccharides having structures 1-8 shown in Figure 1.

In this paper, the collision induced dissociation (CID) spectra for three monosulfated chondroitin sulfates is presented (Figure 1; compounds 2, 3, and 4). These disaccharides produce abundant molecular ions of the type $[M - Na^+]^-$ ($m/z$ 460) and $[M - 2Na^+ + H^+]^-$ ($m/z$ 458) [6], where M is the fully sodiated neutral compound. All negative ion FAB-tandem mass spectrometric analyses were performed using a VG ZAB-HF mass spectrometer in the mass analyzed ion kinetic energy (MIKE) mode. In general helium was used as the collision gas at an analyzer pressure of ca. $5 \times 10^{-9}$ mBar (normal operating pressure ca. $5 \times 10^{-5}$ mBar).

The CID-MIKE spectra arising from the $[M - Na^+]^-$ molecular ion at m/z 460 is unique for each of the three compounds analyzed. That is, the CID-MIKE spectrum of the 2S sulfated compound has a fragment ion at m/z 277 corresponding to the portion of the sugar possessing the sulfate, which is not observed in the CID-MIKE spectra of the 4S or 6S compounds. This is not surprising since the sulfate groups are located on different rings of the disaccharide. Differentiation between the 4S and 6S disaccharides is achieved due to structurally significant fragment ions observed in each spectrum. Most notably are two small fragments containing the sulfate group arising from ring cleavage at m/z 153 for the 4S compound and m/z 139 for the 6S compound. The CID-MIKE spectra arising from the $[M - 2Na^+ + H^+]^-$ molecular ion at m/z 458 also allows for the differentiation between the 2S, 4S, and 6S sulfated disaccharides. Although the same fragment ions are observed in the MIKE spectra of 4S and 6S, differentiation between them is possible due to ion intensity differences. For example, ions at m/z 282 and 300 arise from glycosidic cleavage with and without loss of water and are observed as abundant fragment ions in the MIKE spectra of 4S and 6S. In the CID-MIKE spectrum of 4S, the product ion at m/z 300 is the base fragment ion; loss of water from 300 is a minor fragment ion. For the 6S compound, the product ion at m/z 282 is the base fragment ion observed suggesting that it is formed due to direct bond cleavage.

Figure 2. CID-MIKE spectra of \([M - \text{Na}^+](m/z \ 480)\) and \([M - 2\text{Na}^+ + \text{H}^+]\) (m/z 458) for disaccharides 2S, 4S, and 6S.

Figure 3. Proposed structure of the fragment ions observed in the CID-MIKE spectra of the \([M - 2\text{Na}^+ + \text{H}^+]\) molecular ion.
LOW-MASS IONS PRODUCED FROM PEPTIDES BY HIGH-ENERGY COLLISION-INDUCED DISSOCIATION IN TANDEM MASS SPECTROMETRY.

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High-energy tandem CID mass spectrometry provides a rapid and sensitive means of determining or verifying the amino acid sequences of peptides [1, 2]. Interpretation of a peptide MS/MS spectra relies mainly on the sequence-specific ions present. However, it is important to study all of the ions present in CID spectra, both because of their potential usefulness for interpretation and also as a means of uncovering previously uncharacterized fragmentation modes. The low mass region of peptide CID spectra contains peaks due to immonium and related ions as well as acyl and immonium dipeptide ions [2, 3]. We studied a large number of spectra, concentrating on the mass range 60-300, with a view to eventually incorporating this information into a "second-generation" computer-based scheme for interpretation of peptide CID spectra.

EXPERIMENTAL

MS/MS experiments were carried out on a Kratos Concept IIHH 4-sector instrument of EEBB geometry [4], equipped with an LS1MS source and a multichannel array detector capable of acquiring data simultaneously over a 4% mass window. Precursor ions were generated with an 18 keV Cs+

RESULTS AND DISCUSSION

IImmonium ions. Immonium ions for most of the common amino acids and for many modified residues are prominent in high-energy CID mass spectra [2, 3]. The usefulness for interpretation purposes of the immonium and related ions varies a great deal for different amino acid residues. For example, the cluster of ions produced by Arg is usually unmistakable as the characteristic ions from His, Leu/Ile, Phe, Tyr and Trp. On the other hand, the immonium ions of a few residues, such as Asp and Glu, are sometimes weak or absent. In some cases interferences can occur; for example, proline produces a strong ion at m/z 70, but Arg also generates a strong ion at the same mass. Similarly, the mass 87 ion of Asn is also masked if Arg is present.

Figure 1 shows the low mass region of the tandem CID spectrum of the peptide Ac-Gly-Ile-Gln-Glu-Leu-Tyr-Gly-Ala-Ser-NH2. The spectrum indicates that the peptide must contain He or Leu, Gin or Lys, and Tyr, and that Arg, His, Phe, Pro and Trp must be absent. The spectrum also gives strong evidence that Ser, Glu and Ac-Gly (or Val) are present and that Asn, Cys, Met and Thr are probably absent.

Modified amino acids. The immonium ions produced by modified amino acid residues nearly always produce peaks at the expected mass, namely 27 Da less than the modified residue mass. Acetylated N-terminal residues usually give rise to both acetylated and non-acetylated immonium ion peaks. Residues at the C-terminus of a peptide which have a modified carboxyl group (esters, for example) show only the normal immonium ion. Hexyl esters of aspartate, glutamate and carboxymethylcysteine give the expected immonium ions both with and without the modifying groups. Table 1 is a list of a number of modified residues and the masses of the characteristic positive ion high-energy CID peaks observed when these residues are present.

Dipeptide ions. The peptide CID spectra in Fig. 1 contains several examples of ions related to two adjacent residues in the original peptide. These ions are either internal acyl ions (designated by the dipeptide single-letter code) or immonium ions (designated by the dipeptide single-letter code -28) [5]. Dipeptide ions are often abundant when one of the residues gives rise to a strong immonium ion of its own, and/or when cleavage at one of the residues is favored, such as at the N-terminal side of Pro or Gly. Peaks corresponding to immonium or acyl dipeptide ions for the last two residues in a peptide are rarely or never observed. Dipeptide ion peaks can be particularly useful in interpretation of CID spectra. It is frequently the case that two satisfactory interpretations of a spectrum of an unknown peptide differ by a dipeptide inversion, for example, TATWFSR or TTAWFSR.

REFERENCES

Ac-GIQELYGAS-NH$_2$

![Tandem CID mass spectrum of the peptide Ac-Gly-Ile-Gln-Glu-Leu-Tyr-Gly-Ala-Ser-NH$_2$. Single amino acid immonium ion peaks are marked with their corresponding single letter code. Dipeptide acyl and immonium ion peaks are also indicated (see text) as are the normal sequence ions.](image)

**Figure 1.** Tandem CID mass spectrum of the peptide Ac-Gly-Ile-Gln-Glu-Leu-Tyr-Gly-Ala-Ser-NH$_2$. Single amino acid immonium ion peaks are marked with their corresponding single letter code. Dipeptide acyl and immonium ion peaks are also indicated (see text) as are the normal sequence ions.

<table>
<thead>
<tr>
<th>Amino acid residue</th>
<th>Immonium ion mass</th>
<th>Related ion masses</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Hydroxytyrosine</td>
<td>152</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Methylphenylalanine</td>
<td>134</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e-Acetyllysine</td>
<td>143</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylproline</td>
<td>112</td>
<td>120</td>
<td>N-terminal</td>
</tr>
<tr>
<td>Acetylphenylalanine</td>
<td>162</td>
<td>136</td>
<td>N-terminal</td>
</tr>
<tr>
<td>Acetyltyrosine</td>
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<td>136</td>
<td>N-terminal</td>
</tr>
<tr>
<td>Aminoethylcysteine</td>
<td>120</td>
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<td></td>
</tr>
<tr>
<td>Carboxymethylcysteine</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Acetylglutamate</td>
<td>144</td>
<td>102</td>
<td>N-terminal</td>
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<tr>
<td>Hexyl carboxymethylcysteine</td>
<td>218</td>
<td>134</td>
<td></td>
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<tr>
<td>Hexyl aspartate</td>
<td>172</td>
<td>88</td>
<td>88 weak</td>
</tr>
<tr>
<td>Hexyl glutamate</td>
<td>186</td>
<td>102</td>
<td>102 weak</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine sulfoxide</td>
<td>120</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Norleucine</td>
<td>86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyridylethylcysteine</td>
<td>181</td>
<td>138, 106, 93</td>
<td>106 strong, 181, 138, 93 can be weak</td>
</tr>
<tr>
<td>Pyroglutamic acid</td>
<td>84</td>
<td></td>
<td>N-terminal</td>
</tr>
</tbody>
</table>

**Table 1.** Immonium and other ions from nonstandard amino acid residues

**ACKNOWLEDGEMENTS**
This work was supported by grants from NIH (RR01614) and NSF (DIR 8700766) to A. L. Burlingame.
A COMPARISON OF MS-MS SPECTRA OF PEPTIDES OBTAINED ON 4 SECTOR MASS SPECTROMETERS OF DIFFERENT DESIGN

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INTRODUCTION

A four sector mass spectrometer of BBEB geometry has recently been installed in our laboratory. The instrument was purchased primarily for the characterisation of advanced materials and associated formulations using the technique of FD-MS-MS. However, the problems associated with the combination of field desorption with tandem mass spectrometry (relatively weak ion currents and ion beams which can have a tendency to be somewhat transient in nature) have meant that this potentially powerful combination has not enjoyed the success that might otherwise have been the case. Consequently, there has been little applications data published in this area and so, as a way of comparing the performance of our system with other instrumentation of its type, we have evaluated the performance of the system using LSIMS in the peptide analysis area. A series of peptides have been examined on our four sector system (the VG ZAB-T) and compared with data obtained on a Kratos four sector mass spectrometer (Concept IIHH). The instruments were, as far as is practically possible, tuned in a consistent, reproducible manner to allow a sensible comparison of the resulting product ion spectra to be undertaken. Product ion spectra which compare and contrast the resulting data which have been obtained for Bradykinin are compared in this poster.

EXPERIMENTAL

The two four sector mass spectrometers utilised in this study were the VG ZAB-T and the Kratos Concept IIHH and these were prepared for operation in product ion mode by firstly tuning the instruments using CsI analysed in LSIMS mode. The tuning procedure used was carried out in as systematic a way as possible and the following tuning protocol was used:

(a) Select m/z 1692 peak of CsI using MS1
(b) Attenuate the beam with collision gas (25% transmission for Ar, 30% transmission for He)
(c) Tune to obtain optimum intensity for parent ion and a range of fragment ions

This procedure was followed using an 8kV source accelerating voltage and with the collision cell floated at 4kV (ie 4kV collision energy). Having obtained CsI product ion data, then the peptide Bradykinin was analysed in LSIMS mode using 4kV collisions and the Ar/He attenuation conditions indicated above.

RESULTS AND DISCUSSION

(a) CsI data

Table 1 provides a comparison of the product ion intensities obtained for product ions produced by high energy (4kV) CID of m/z 1692 of CsI.
Table 1: CsI (m/z 1692) Product Ion Intensities Relative to Parent Ion Intensity at Optimum Tuning Conditions

<table>
<thead>
<tr>
<th></th>
<th>912</th>
<th>393</th>
<th>133</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZAB-T</td>
<td>10I</td>
<td>40I</td>
<td>1I</td>
</tr>
<tr>
<td>Concept</td>
<td>12I</td>
<td>24I</td>
<td>5I</td>
</tr>
</tbody>
</table>

These data, which show similar product ion yields at m/z 912, do begin to show interesting differences in terms of the relative ion intensities at m/z 133/393.

(b) Bradykinin

The product ion data obtained on the VG and Kratos instruments for Bradykinin appear to show some interesting differences (in terms of the overall appearance of the spectra) in the product ion spectra observed. Table 2 is a comparison of the relative product ion yields which were observed during the analysis of Bradykinin by LSIMS-MS-MS (normalised to the most intense product ion in the three ions considered here).

Table 2: Bradykinin Relative Product Ion Yield Comparison

<table>
<thead>
<tr>
<th>Instrument</th>
<th>CID Gas</th>
<th>70/BP*</th>
<th>527/BP</th>
<th>904/BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZAB-T</td>
<td>Ar</td>
<td>35I</td>
<td>100I</td>
<td>61I</td>
</tr>
<tr>
<td>ZAB-T</td>
<td>He</td>
<td>7I</td>
<td>49I</td>
<td>100I</td>
</tr>
<tr>
<td>Concept</td>
<td>Ar</td>
<td>100I</td>
<td>12I</td>
<td>4I</td>
</tr>
<tr>
<td>Concept</td>
<td>He</td>
<td>100I</td>
<td>15I</td>
<td>11I</td>
</tr>
</tbody>
</table>

*BP indicates the base peak in the three product ions considered here.

Table 2 highlights a number of issues which merit comment. The two sets of data, despite the systematic approach to the experiments undertaken, are quite different. Of particular interest is the much higher yield of product ions observed in the Concept spectra at low (<m/z 150) mass. It is not clear at this point as to the reasons for this difference but it is presumably caused by a complex combination of collision cell design, extraction focus conditions, etc. Both sets of data do allow sequence specific information to be generated, and further work is necessary to determine if other sets of collision conditions on the two instruments are more appropriate to this type of analysis.

The initial conclusions from this work, in terms of the type of product ion spectra generated, have given us much confidence regarding the quality of data which we might expect to generate using the FD-MS-MS technique. This work has now begun in our laboratory and is the subject of other presentations at the 1991 ASMS conference.
An important advantage of tandem mass spectrometry (MS/MS) is its ability to isolate an ion from a complex mixture and subsequently obtain its collisionally activated spectrum. In this way, mixture components can be analyzed directly and their chemical structures can be elucidated without interferences. Comparison of an unknown spectrum with a library of known spectra is considered to be an effective method to identify chemical structures. The technique is simple, reliable, and relatively fast as compared to other computer-automated methods such as pattern recognition and heuristic interpretation. The successful operation of a library system depends on the quality of the comparison parameters used in the search, on the choice of standard conditions for spectral acquisition and on the degree of control of the experimental parameters affecting the reproducibility of the spectra. Moreover, the ability to reproduce similar enough spectra enabling the use of a common library, with mass spectrometers in different laboratories, is very important for the success of the technique.

In our laboratory, we have examined high energy (KeV) CAD processes and created a library of MIKES/CA spectra using the energy centroid, peak area, and peak width as parameters for structure identification purposes. Since several experimental factors such as collision gas, beam attenuation, collision energy, instrument geometry and others can affect the efficiency of the MS/MS library search we have undertaken a systematic study of the optimal experimental conditions under which the system would give reliable results. The instruments used in this study for the acquisition of MIKES/CA spectra were a Kratos MS50-TC triple sector mass spectrometer of EBE geometry, and a VG Autospec-C hybrid mass spectrometer of EBEqQ geometry. In chemical ionization the reactant gas was isobutane, and samples were introduced via a direct insertion probe. Samples in the FAB ionization mode were dissolved in glycerol, and were bombarded with a 14KeV caesium ion beam. Treatment and comparison of energy spectra were performed using an automated MIKES/CA data system (1). Instrumental conditions for the use of the library were calibrated using n-decane in El, glycerol in CI and FAB/LSIMS, and ethylene glycol in thermospray. The specific conditions of the El source for accumulation of kinetic energy MS/MS spectra are: 70eV electron energy, 200° C source temperature, and \(10^{-6}\)Torr source pressure. The ion repeller voltage was optimized for maximum ion extraction. Precursor ion selection is preferred to be conducted under high mass resolution conditions to eliminate isobaric ion contributions in mixture analysis. In the activation step, helium has been chosen as the appropriate target gas due to minimization of scattering and charge exchange effects, and production of good ion intensities. The standard collision energy of 8keV was selected for maximum sensitivity and convenience. However, the range 4000-8000 keV is acceptable for use of the library. The energy resolution interval =500-800 has been selected as the optimal resolution range for acceptable peak separation and maximum sensitivity.

The reproducibility of MIKES/CA spectra, examined using n-decane as a reference compound, was found to be within acceptable limits identification. Reproducibility for consecutive scans ranged between ±0.9% and ±2.9% of fragment ion abundances. Errors in the encoding of weak peaks were reduced using spectral averaging techniques and appropriate digital filter methods. Spectra obtained for an energy resolution interval = 600-2700 (13eV-3eV main beam width at 10% height) were found to be similar and showed a decrease in S/N at higher resolutions. Experiments conducted on different days were found to produce spectra with reproducibility errors for the various ion abundances within the ±0.5% and ±2.4% range.

The dependence of fragmentation patterns on the target gas pressure has been examined using several compounds of common organic chemical classes. Fragmentation patterns can change drastically within the 0%-30% beam attenuation interval (FIGURE 1, FIGURE 2) and small pressure changes can produce considerably different spectra. Beam attenuations higher than 70% although they usually produce extensive fragmentation, are associated with weaker MIKES/CA spectra, due to the increased probability of scattering, and the other competing collisional processes. Therefore, a 50% main beam attenuation has been chosen as a representative value in the generally accepted beam attenuation interval 30%-70% where fragmentation patterns are relatively stable and sufficient fragments are present allowing good fingerprinting.

A good overall similarity between spectra was obtained with two tandem mass spectrometers of similar geometry under the established experimental conditions for several classes of chemical compounds. As an example, the data presented in Figure 3 indicate that the spectra generated by different mass spectrometers are fairly similar despite instrumental differences. Thus, a general application of the MS/MS search approach is possible, using a common data base of MIKES/CA spectra for comparison and identification of
spectra acquired with different mass spectrometers.

In the process of evaluation of the search approach, several dipeptides were used to investigate the effect of the ionization method on the fragmentation pattern of the MS/MS spectra. As an example, the CI and FAB mass spectra of the MH⁺ ion (m/z 189) of the Glycyl-L-Leucine dipeptide, are considered. The CI and FAB MS/MS spectra of the m/z 189 protonated dipeptide shown in Figure 4, are very similar. The CI and FAB MIKES/CA spectra of at least 14 other dipeptides have been acquired and found to be identical within experimental reproducibility limits. This observation indicates that under the employed conditions, the CI and FAB ionization sources can lead to the formation of the same precursor ion structure (or a mixture of structures). An important implication of ionization method-independent MS/MS spectra of protonated species is the fact that a wider choice of ionization sources can be used without the need of multiple data bases.

REFERENCES

Four-sector tandem mass spectrometry is proving extremely useful for providing sequence information for peptides. Daughter spectra of large numbers of peptides may be generated in a relatively short time-frame. However, efficient interpretation of the data from high energy collisions remains one of the obstacles to rapid sequencing by mass spectrometry, due mainly to the complexity of the fragmentations. We have developed a derivatization procedure that produces simplified, predictable fragmentation of ions produced by FAB to permit more rapid and accurate data interpretation.

A fixed positive charge is attached to the peptide by (a) acylation with iodoacetic anhydride at pH 6, which selectively modifies the N-terminal α-amino group (1), and (b) subsequent addition of a dimethylalkyl amine at pH 8 to form a quaternary ammonium group. The first step exploits the pKa differences between the α-amino group and any lysine side-chain ε-amino groups. The second step yields improved spectra over previous derivatives (2) in which step (b) was addition of thiocholine. The quaternary ammonium derivatives have also been prepared by Vath and Biemann using gas-phase methodology (3). A related approach, developed by Wanger et al., utilizes an ethyltriphenylphosphonium moiety to direct fragmentation (4).

The derivatives in this study are prepared as follows: 0.1-2 nmol of peptide is dissolved in 12 µl of 0.3 M 2-[N-morpholino] ethanesulfonic acid (MES), pH 6. The solution is cooled to 0°C and 5 µl of 0.01 M iodoacetic anhydride in dry THF is added. The solution is vortexed, maintained at 0°C for 5 min, and then allowed to return to ambient temperature. To the iodoacylpeptide is added 5 µl of either 12.5% trimethylamine, 0.5 M dimethylhexylamine, or 0.5 M dimethyloctylamine. The solution is vortexed and heated to 37°C for 2 hrs. The derivatized peptide is purified by reversed-phase HPLC. Mass spectra were obtained with a JEOL JMS-HX110HF/HX110HF tandem mass spectrometer using a 3-inch JEOL array detector (9.5% simultaneous mass range). Peptide ions were produced by 10 keV Cs⁺ primary ions, using m-nitrobenzyl alcohol or monothioglycerol as the matrix, and accelerated to 10 keV. The collision gas was helium, and the collision cell was operated at 6 kV.

The amine alkyl chain length was varied from C1 to C8 for the hexapeptide FGAKQA. Dimethyloctylamine was found to produce the best parent ion signals due to its higher surface activity in the matrix. The simplification of the daughter spectrum of the derivatized peptide is evident by comparing Fig. 1, the daughter spectrum of 100 pmol of an undervatized tryptic fragment (T4) from recombinant human growth hormone, with Fig. 2, the corresponding dimethyloctylaminoacetyl (DMOA-Ac) peptide (40 pmol). The sequence of the peptide is LHQLAFDTYQEFEEAYIPK. The spectrum of the undervatized peptide exhibits partial series of aₙ, bₙ, cₙ, dₙ, eₙ, fₙ, gₙ, hₙ, and m+n+1 ions, plus numerous internal fragments at low mass. The determination of the sequence from this spectrum alone would be difficult. In contrast, the spectrum in Fig. 2 shows a complete set of aₙ ions from which the entire sequence of this 19-residue peptide can be derived. Moreover, a strong series of dₙ ions is observed which permits distinction between Ile and Leu residues and also provide additional confirmation of the sequence. A minor cₙ ion series is also observed, but few bₙ ions are found. By limiting the fragmentations primarily to three ion series, the peaks observed are stronger than those from the undervatized peptide because the available daughter ion current is distributed among fewer masses.

In summary, this derivatization procedure (a) enhances interpretation of CAD spectra by yielding simpler fragmentation, (b) enhances signal strength by concentrating the parent ion abundance in fewer fragmentation pathways, (c) permits distinction of Ile and Leu by exhibiting abundant dₙ ions, and (d) lowers the necessary daughter ion resolution by producing fewer, well separated peaks.
FAST ATOM BOMBARDMENT AND TANDEM MASS SPECTROMETRY OF QUATERNARY PYRIDINIUM SALT-TYPE TRYPTOPHAN DERIVATIVES

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1Center for Drug Discovery and 2Department of Pharmacology and Therapeutics, University of Florida, Gainesville, FL 32610; 3Pharmatec, Inc., Alachua, FL 32615; 4Mount Sinai School of Medicine, New York, N.Y. 10029.

Fast atom bombardment (FAB) and liquid secondary-ion mass spectrometry (LSIM) provide routine means of efficiently sampling quaternary salt-type compounds for subsequent mass analysis. Optimally, these desorption ionization techniques generate intact ionic species (the molecular cation in positive-ion mode) as well as structure-related fragment ions from the sample dissolved in a suitable liquid matrix upon the impact of the keV-energy atom/ion beam. The desorption of closed-shell quaternary ions is of special interest, since usually no apparent chemical interaction leading to proton/cation attachment from the constituents of the matrix can be observed. FAB and LSIMS may not actually be referred as "soft" ionization techniques, since abundant fragment ions analogous to those observed in EI or CI mass spectra are commonly formed. Fragmentation pathways for the FAB technique can also be elucidated with the same rigor as the EI/CI processes.

We have recently synthesized [1,2] quaternary pyridinium salt-type derivatives of tryptophan (α-amino-1-indolepropionic acid) in which the structure of the organic cation varies with respect to both the substituents and the distance of the quaternary nitrogen from the indole/carboxyl portion. In structure 1, the α-amino group of the parent compound is transformed into the quaternary nitrogen of the pyridinium cation. Structure 2 represents derivative in which trigonellyl, a pyridinium-type acyl group, is used to attach the charged portion to tryptophan esters. Compound 3 is also an amide; the quaternary nitrogen is, however, separated from the acyl carbonyl by -CH2-"spacer." The localization of the charge on a particular structure has especial significance for the mechanistic interpretation of fragmentation pathways of organic ions. Unlike large, highly functionalized molecules where the site of the positive charge arising from proton or cation attachment may not be clear, the title structures provide unambiguously localized charge—the quaternary nitrogen. In this presentation, we elucidate fragmentation processes of these pyridinium ions occurring under FAB conditions. Accurate mass measurement, isotope labeling, and tandem mass spectrometry have been used to gain insight into the origin and possible structure of the ions of interest.

FAB mass spectra were recorded on a MS80RFA/DS90 instrument (Kratos Analytical, Manchester, U.K.). The FAB gun, an Ion Tech Ltd. (Teddington, Middlesex, U.K.) FAB11NF saddle-field source, was operated with research-grade xenon at 8 keV beam energy and 1 mA current. Sample introduction was accomplished via placing 1-5 µg of analyte onto the copper probe tip, and then mixing with 1-2 µL of liquid matrix (glycerol or thioglycerol). CID and tandem mass spectrometry studies were carried out on a Trio-3 (VG Instruments, Manchester, U.K.) triple quadrupole instrument. The samples were dissolved in glycerol, and FAB experiments were performed with a xenon gun operated at 8 kV and 2 mA. All MS/MS experiments (daughter and parent ion scans) were recorded using nitrogen as collision gas at a pressure of 2-6 millitorr in the RF-only quadrupole. Collision energy was 5-15 eV, adjusted to give maximum intensities for the particular transitions studied.

For the pyridinium ions involved in our study, the FAB ions obtainable from ion source reactions and the CID experiments have revealed complex, structure-dependent fragmentation patterns. A variety of cleavage reactions apparently independent of the localized charge and frequently taking place at charge remote sites of the structure have been identified even under experimental conditions that favor reactions with low energy barrier. Though the extent of such reactions may be largely dependent on the arrangement of the functional groups within the ions involved, certain uniformity of the governing principles of fragmentation has been demonstrated by keeping portions of the structure, intact. Remarkably, ions with new charge sites formed during the homolytic cleavage reaction may dominate as product species in the respective mass spectra over the ones that retain the pyridinium structure. Further studies are to reveal mechanistic details on the underlying processes, and to extend our knowledge on the chemistry of these closed-shell ions. The following fragmentation pathways have been elucidated for the selected quaternary pyridinium salt-type tryptophan derivatives:
References


INVESTIGATION OF CONFORMATIONAL EFFECTS ON FRAGMENTATION OF N-PROTECTED TRIPEPTIDES BY MS/MS

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INTRODUCTION

Determination of conformation of peptides by mass spectrometry has become important for the prediction of secondary structure of oligopeptides. To explore the relationship between intramolecular hydrogen bonding caused by side chain interactions and intensity of products formed, two N-protected tripeptides, N-Cbz-Gly-Leu-Arg-NH₂ and N-Cbz-Gly-Pro-Arg-NH₂ were studied using LSIMS MS/MS on a hybrid tandem mass spectrometer. The differential loss of benzyl alcohol (106 u) from the protonated molecular ion of the two peptides and the formation of significant other products were studied under unimolecular decomposition and CAD conditions as a function of collision energy and gas pressure.

RESULTS AND DISCUSSION

MS/MS relative ion intensities were found to differ dramatically from conventional MS-I full scan results. For example, contrary to full scan data, the [MH - 108]+ ion at m/z 354 in the MS/MS spectra of Cbz-Gly-Pro-Arg-NH₂ is an intense product ion, often the most intense observed, under all collision energies and gas pressures investigated, having abundances ranging from 7 to 17%; however, the corresponding ion for Cbz-Gly-Leu-Arg-NH₂ at m/z 370 never reaches intensities greater than 4% relative to the sum of all products (Figure 1). The intensities of these ions, as well as other high mass products, decreases at the Eolation approaches 50 eV, with a corresponding increase in intensity of lower mass ions, such as m/z 70, 86 and 91, under CAD conditions, with the effect being more pronounced at higher Ar pressures (Table 1). Another major difference in the MS/MS behavior of the two peptides is that higher intensity lower mass fragment ions are observed only from the leucine peptide (Figure 2). Higher collision energies and gas pressures are required to induce the production of lower mass fragments for the proline peptide, suggesting that the extended time in the MS/MS experiments versus the time in the source allows the proline peptide to assume a more stable conformation through intermolecular hydrogen bonding between side chains because of the nature of the proline ring.

EXPERIMENTAL

Cbz-Gly-Leu-Arg-NH₂ and Cbz-Gly-Pro-Arg-NH₂ were synthesized by reacting Cbz-Gly-Leu-OH and Cbz-Gly-Pro-OH, respectively, with H-Arg-NH₂ using DCC as a coupling reagent. The peptides were purified by HPLC and dissolved in methanol.

LSIMS mass spectra were obtained on a VG 70-250 SEQ instrument (EBQQ), 8kV in MS-I. A cesium ion gun was operated at 25 kV (1.5 uA emission), with glycerol matrix and 5-µl sample volumes. Product ion spectra were acquired in the multi-channel analysis (MCA) mode by scanning from m/z 50 to 500 with the accumulation of 15 scans. Four collision energies were chosen ranging from 10 to 50 eV with low pressure CAD conditions at Ar pressures of 10⁻⁷-10⁻⁶ mbar (primary beam attenuation by 10%) and high pressure CAD conditions at 5 X 10⁻⁵ mbar (by 90%).

REFERENCES

The 39th ASMS Conference on Mass Spectrometry and Allied Topics

**HIGH PRESSURE CAD OF [PMH]⁺**

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**HIGH PRESSURE CAD OF [IMH]⁺**

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<td>463</td>
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* Base Peak
Many aromatic molecules have strong absorption bands in the UV which are rather broad at room temperature; the ionization potentials for many such polyatomics are also often less than 9 eV. Therefore, they can be ionized with high efficiency by resonance enhanced two-photon ionization (R2PI) using the frequency-quadrupled output of the Nd:YAG laser at 266 nm (4.7 eV). Ionization selectivity for polyatomics is necessarily limited when fixed-wavelength R2PI is used, however. Although improvements in ionization selectivity can be achieved through the use of tunable UV laser radiation, limitations exist on the optical selectivity of tunable R2PI attainable at room temperature due to the thermal population of excited rovibronic states. Moreover, in some situations such as rapid screening of complex mixtures for compound class, it may be advantageous to use a relatively nonselective ionization method in conjunction with another technique which enhances the analytical specificity. Because the degree of fragmentation accompanying R2PI is a function of laser power, mass spectrometric analysis of such fragments also can often improve the specificity of the overall analysis. However, in the case of organic mixture analysis, the complexity of R2PI/fragmentation mass spectra may compromise compound identification. MS/MS can also enhance the specificity of analysis over that provided by a single stage of mass spectrometry. Although R2PI/fragmentation and MS/MS can yield complementary data, situations may often occur in which the spectra reveal no unique structural information. Nevertheless, the combination of R2PI and MS/MS can still provide an advantage over the use of R2PI alone.
R2PI/fragmentation mass spectra obtained in the ITMS for ethylbenzene, propylbenzene, N,N-dimethylaniline, and N-ethylaniline are shown in Figures 1-4, respectively. The R2PI-MS/MS spectra for each of the sample compounds contain major product ions corresponding to those major fragment ions seen in the R2PI/fragmentation spectra. On the basis of the above results, combining MS/MS with R2PI appears to provide little advantage over the use of R2PI alone for the analysis of pure compounds. However, the effectiveness of R2PI-MS/MS compared with R2PI/fragmentation for mixture analysis can be demonstrated using a mixture of the above compounds.

The molecular ion signals at m/z values 106 and 120 in Figure 5a, obtained using unfocused laser radiation, indicate the presence of ethylbenzene and propylbenzene, respectively. However, the m/z 121 ion signal could be due to either N,N-dimethylaniline, N-ethylaniline, or both compounds. Although the increase in the m/z 120 signal in Figure 5b, acquired using focused laser radiation, suggests that N,N-dimethylaniline is present, such an effect could be attributed to propylbenzene. In any case, the presence of N-ethylaniline cannot be confirmed because its major fragment ion at m/z 106 coincides with the molecular ion of ethylbenzene. Therefore, the origin of the ion signal at m/z 121 can not be confirmed. Thus, despite the fact that individual R2PI/fragmentation mass spectra for the components comprising a sample are unique, mixture analysis can be problematic when fragment ions are indistinguishable from molecular ions.

Tandem mass spectrometry in conjunction with R2PI can enhance specificity in such circumstances. An MS/MS spectrum (Figure 6) of the m/z 121 molecular ion from the same mixture was obtained using unfocused laser radiation and the same CID parameters previously used for pure component spectra. As seen in the spectrum, the major product ions are at m/z values 106 and 120. Comparison with pure component MS/MS spectra reveals that the m/z 106 and 120 product ions correspond to the N-ethylaniline (M-CH₃)* and N,N-dimethylaniline (M-H)* ions, respectively. Given this MS/MS structural information and the molecular ion data obtained from the low power R2PI mass spectrum (Figure 5a), it can then be deduced that the sample is comprised of all four components. Given any combination of mixtures generated from the same suite of compounds, similar reasoning could be used to deduce the composition.
APPLICATION OF SUBSTRUCTURE IDENTIFICATION RULES TO MS\textsuperscript{n} SPECTRA OBTAINED ON AN ION TRAP MASS SPECTROMETER

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Oak Ridge National Laboratory, P.O. Box 2008
Oak Ridge, TN 37919-6365

Empirically derived rules have been generated by the MAPS software for use in identifying molecular structures in unknown compounds from MS/MS spectra [1]. The rules were based on a training set of CID product spectra obtained on a triple quadrupole mass spectrometer (TQMS) under single collision conditions. The TQMS was used for acquisition of the training set MS/MS spectra because the instrument was capable of rapidly acquiring the CID reference spectra. The ion trap mass spectrometer (ITMS), however, has the advantage of better sensitivity and CID efficiency compared to the TQMS. Therefore, a study of the applicability of rules generated from the TQMS reference spectra to MS/MS and MS\textsuperscript{n} spectra acquired on the ITMS was performed.

The MAPS rule for the phenothiazine substructure was used as a model for this study. In particular, the CID products obtained for the m/z 198 ion (the intact phenothiazine ring) were investigated. The CID products observed in the single collision TQMS spectrum (1% threshold) are m/z 197 (loss of H), m/z 171 (loss of HCN), and m/z 154 (loss of CS). The m/z 45 (HCS+), m/z 166 (C12H8N+), and m/z 127 (C10H7+) ions were also observed in this spectrum upon the use of a blowup factor. The MAPS rule generated using multiple collision CID spectra included m/z 197, m/z 171, m/z 166, m/z 154, m/z 128, m/z 127 and m/z 45 (using the 1% threshold). The ITMS MS/MS spectrum of the m/z 198 ion derived from phenothiazine is shown in Figure 1. Note that the spectrum shown was acquired with a q value of 0.45, a value chosen to maximize CID efficiency (approx. 100%). This q value was too high to observe the m/z 45 product ion. A separate spectrum was taken using a q value of 0.18. The m/z 45 was not observed for the m/z 198 precursor ion of phenothiazine or any of the eight other standards listed in Table 1. This ion is actually the most abundant CID product ion in the multiple collision TQMS spectrum. The m/z 166 CID product ion was also missing from two of the nine phenothiazine standards.

Application of the single collision MAPS rules to the nine phenothiazine standards yielded nine identifications of the presence of the phenothiazine substructure while only seven identifications were made using the multiple collision rule. The difference in these results was the missing m/z 166 and m/z 45 product ions in the ITMS spectra (MAPS rules do not utilize relative intensities, just the presence of specified spectral features). Ion activation in the ion trap proceeds by successive collisions with relatively small increments of energy deposition compared to the TQMS experiment. Thus, xenon was added to the He buffer gas (approximately 1:1 for a total pressure of 1.2 x 10\textsuperscript{-4} torr) in an effort to increase the energy deposited per collision in the ITMS. The MS/MS spectra obtained with (top) and without (bottom) resonant excitation are shown in Figure 2. The ions observed in the bottom spectrum appear because of incomplete isolation of the m/z 198 precursor ion (done to maximize m/z 198 signal before resonant excitation). The top spectrum of Figure 2 represents the largest m/z 45 signal observed (approximately 3% of the base peak m/z 198 and approximately 0.8% of the original m/z 198 abundance). Increasing tickle time or tickle voltage leads to decreasing m/z 45 signal probably due to ejection of the precursor ion before dissociation can occur. The 5 x 10\textsuperscript{-5} torr pressure of xenon was also optimal to observe the 198 -> 45 CID reaction.

**TABLE 1**

<table>
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<tr>
<th>Compound Name</th>
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<tr>
<td>phenothiazine</td>
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<tr>
<td>promazine</td>
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<td>trimipromazine</td>
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</tr>
<tr>
<td>trifluromazine</td>
<td>P F P F P P P P A</td>
</tr>
<tr>
<td>thiole blue</td>
<td>P F P F P P P P A</td>
</tr>
</tbody>
</table>

P: Present in ITMS spectrum  A: Absent in ITMS spectrum

773
Since the q parameter is such a critical parameter in CID by resonant excitation in the ITMS, another ion
activation method such as photodissociation, which does not lose efficiency as the q parameter decreases (for
values set to store the product ions), may yield better results. The challenge in utilizing photodissociation in the
ion trap lies in dissociating the selected ion without ionizing neutral molecules.

1 K.J. Hart, A.P. Wade, B.N. Nourse and C.G. Enke, JASMS, accepted.

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Laboratory Cooperative Postgraduate Research Training Program administered by Oak Ridge Associated
Universities.

FIGURE 2

ITMS SPECTRUM, tickle: q=0.18; 220 mV; 10 ms
Helium and Xenon Bath Gas Mixture (1:1)
p (total) = 1.2 x 10^-6 torr

TICKLE ON

TICKLE OFF

(incomplete isolation of m/z 188)
AN APPROACH FOR THE DESIGN OF MS\textsuperscript{n} EXPERIMENTS ON A MULTISECTOR HYBRID SPECTROMETER OF EBEqQ GEOMETRY.

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Regional Center for Mass Spectrometry,
Department of Chemistry, University of Montreal, Montreal, Canada, H3C 3J7.

 Since the accelerating voltage was first scanned to analyse metastable ions, a great deal of work has gone into the study of fragments formed in field free regions. MS\textsuperscript{3} type scans, performed on an instrument with three mass analysers of BEqQ geometry, have been shown to exhibit a distinct improvement in selectivity over the more common MS\textsuperscript{2} type scans (1). Using a four sector instrument (ZAB-4F), MS\textsuperscript{4} and MS\textsuperscript{5} type experiments were not only proven feasible, but also useful in the structural elucidation of biopolymers (2). The development of more complex multisector mass spectrometers with high performance computer systems enables tandem mass spectrometry experiments to be conducted more easily and more reliably, opening the door to a multitude of possible MS\textsuperscript{n} scans, some involving several consecutive fragmentations (n=3,4,5). A new nomenclature, first introduced to describe the MS\textsuperscript{3} type scans, allows all the scans theoretically possible on an instrument to be easily defined according to the function of the scan rather than the scanning method. These scanning modes increase exponentially with the number of analysers in a given system. Since programming all the scans would be a near impossible task, considering that scans with a functional relation between two masses can be individually tailored, a new approach is suggested that allows the user to program any desired linked scan. The concept of this approach is to translate scan equations, typed by the user into the computer, into the correct commands to execute the scan through the use of software controlled DACs. Examples are given to show the feasibility and the flexibility of this approach for multisector mass spectrometers.

Until recently, typical linked scans (B/E, B\textsuperscript{2}/E, neutral loss) were available on most magnetic sector mass spectrometers only as pre-programmed functions. While for linked scans pre-programmed functions simplify the work involved in performing such scans, they limit the user by not allowing him to investigate special cases, which require the design of customized experiments. The scan modes which are possible on an instrument can be obtained by consideration of the fundamental scans, the functional, and total scans that can be conducted on a four sector spectrometer. As theoretically calculated by Cooks et al., the total number of scans for a four sector geometry is 74 scan modes, if all MS\textsuperscript{n} (n=0-4) experiments are considered. To allow the design of all possible MS\textsuperscript{n} experiments on the instrument and in keeping with the modern trend of user friendly programs and menu-type displays, the new approach was developed based on the use of a graphical matrix. The concept of this matrix is applicable to an instrument of any geometry. In this case, a VG Autospec-Q double focussing instrument of EBEqQ geometry was used. The instrument comprises two collision cells, located in FF1, FF3 and the rf-only quadrupole, and since the three sectors, the two quadrupoles, and the accelerating voltage are each controled by their individual DACs, it is thus well suited to the development of such a matrix.

The approach considers linked scans as equations in which each sector involved is represented as a variable in the mathematical equation. As seen below, the matrix consists of two main sections: the top row is used to select which sector should scan or not scan, and the lower grid is used to enter the equation that describes the relationship between two sectors (Figure 1). The two sectors linked by the relationship are selected according to the intersection of their respective row and column. By first selecting the sectors involved in the scan, the user can enter an equation that describes the link between the sectors. An interpreter will evaluate the equation and determine whether it is mathematically correct. Existing menus can be used to enter the missing values, necessary to calculate the scan parameters, such as the accelerating voltage, the mass range of the scan, the mass of the parent, daughter or neutral fragment, and other instrument parameters. For example, in order to set a B/E linked scan experiment, E1, M, and E2 would be set to the scan mode and E1 and E2 would be linked together and to the magnet as indicated in the matrix in Figure 1 by the relations R1, R2 and R3, which are defined as shown. In those relations, m represents the parent mass and M the field corresponding to the daughter ions. As a comparative test, a daughter ion scan of m/z 122\textsuperscript{+} in ethylbenzoate was conducted using conventional software and using the "link-matrix". The results obtained with the "link-matrix" are shown in Figure 2, and are comparable to those obtained with pre-programmed functions for the same scan.

One of the experimental limitations of MS\textsuperscript{n} experiments is the sensitivity with which these experiments can be conducted, if several fragmentations are involved. In order to investigate this aspect, MS\textsuperscript{5} type experiments were conducted to evaluate the practicality of the approach.
In these experiments the daughter ion spectra of m/z 77⁺ in the spectrum of ethylbenzoate were obtained by scanning the last analyser (Q). The parent ion was selected by increasing the number of fragmentations after the source leading to its formation from the molecular ion of ethylbenzoate. The results obtained in MS⁴ and MS⁵ experiments are shown in Figures 3 and 4. It can be observed that the S/N ratios are quite acceptable for this type of experiments. The acquisition times, that range from 150 to 600 seconds compare well with published data, in which the times ranged from 30 minutes to 3 hours for similar experiments. Thus, the use of more recent computerized data acquisition modes makes these experiments feasible, and the results demonstrate the possibility of performing multiple fragmentation experiments in a reasonable amount of time. Therefore, it would appear that the approach which is presented is worth pursuing to explore the types of MSⁿ that can be conducted on this instrument or other types of multisection spectrometers.

Although not all 74 different scans theoretically possible on the EBEqQ geometry will have analytical applications, the instrument is shown capable of performing even the ones involving multiple fragmentations. Therefore, if the matrix can correctly interpret scan equations for customized experiments, and providing the equations are correct from the point of view of a linked scan, then the instrument should execute the instructions and produce a spectrum. This new approach effectively allows the user to design an experiment for a particular application. The greatest advantage of the "link-matrix" is its versatility, especially when performing scans with a functional relation between masses.

REFERENCES
The development of techniques allowing the introduction of liquid samples has considerably increased the range of compounds that can be analysed by mass spectrometry. Although substantial efforts have been devoted to developing interfaces in order to couple liquid chromatography to mass spectrometry, there are several other applications that can benefit from such liquid introduction techniques. Among these applications that require continuous introduction of the sample, the identification of organic compounds by techniques of MS or MS/MS and real time monitoring of chemical reactions occurring in solution can be taken as examples. A survey of the existing liquid introduction systems shows that DLI systems using a simple capillary to transfer the liquid sample into the ion source of the mass spectrometer are the most appealing. These systems are inexpensive, simple, and can be easily adapted to most magnetic sector and quadrupole mass spectrometers. Examination of the actual configuration of most GC/MS systems using direct coupling immediately leads to the conclusion that they could be used for capillary DLI interfaces with thermal nebulization. In this perspective the feasibility of using the direct coupling GC/MS interface of a magnetic sector mass spectrometer to introduce liquid samples was evaluated.

The instruments used in this study were a VG TRIO-1 quadrupole mass spectrometer equipped with differential pumping and a VG-AutoSpec-Q magnetic-sector hybrid mass spectrometer of EBEqQ geometry. The TRIO-1 was slightly modified by the addition of a high vacuum gauge and the source was slightly altered to increase its pumping speed and sensitivity. The VG-AutoSpec-Q was used without modification. The DLI experiments were done by directly introducing a 50 μm I.D. fused-silica transfer capillary into the EI/CI source of either mass spectrometers. On both instruments, the transfer capillary was introduced by the GC oven through the conventional GC/MS Interface where it replaced the GC capillary column and was adjusted into the ion source exactly like the GC column. The capillary was directly connected at the other end to a microinj ector valve (60nL) through which the solution containing the sample was introduced. The transfer capillary was heated over the length of the interface and maintained at the optimal temperature on both spectrometers using the standard GC/MS interface heaters. The chemical ionization source was operated using the DLI mobile phase as a reagent gas.

The temperature of the interface is an important factor that influence the performance of the DLI systems using capillary thermal nebulisers. Since the temperature of the capillary governs the rate and stability of vaporisation of the mobile phase, the effect of interface temperature was studied in relation to the stability of the systems. In the range of 180-220 °C the interface showed excellent stability in the order of few percent. This can be seen by examination of the TIC shown on Figure 1. Outside this range partial to severe instability was encountered on both sides of this temperature range. Another Important factor that affects the stability and the operating conditions is the flow rate. Its value has to be such that the thermal input through the interface is sufficient to vaporize the mobile phase while the pressure within the chemical ionization source is adequate to maintain ionization and sensitivity. The range of flows corresponding to stable operating conditions found over six months of operation is between 0.5 to 1.6 μL/min on the Autospec-Q and between 0.5 to 2.0 μL/min on the TRIO-1. With these flows, the major ion in the plasma at the lower pressures (<4×10^-5 torr) corresponds to protonated acetonitrile at m/z 42, while the protonated dimer at m/z 83 is small. As the pressure is increased, the intensity of the dimer increases and it becomes the most important ion. Ions corresponding to H2O+ and HgO+ at m/z 18 and 19 have weaker intensities than those related to acetonitrile.

Inter instrument variation between a magnetic sector and a quadrupole mass spectrometer can be evaluated by comparison of the spectrum of ibuprofen obtained on both instruments (Fig.2). The results indicate that the interfaces performs similarly on different types of instruments. The reproducibility for the Autospec-Q can be observed on Figure 3 where several injections of ibuprofen have been made consecutively. This simple, transportable DLI interface can be used advantageously not only for LC/MS (1) analysis but also for MS and MS/MS experiments. The stability of the system facilitates exact mass measurement experiments using the continuous introduction of reference compound with the analyte of interest or by allowing the introduction of the analyte by injection in the mobile phase. Exact mass measurements can be done with an accuracy of 10 ppm with the present system. Since the system offers good operating stability and can produce stable and persistant ion beams it also allows tandem mass spectrometry experiments to be performed at low levels of concentrations. Figure 4 shows the CA spectrum of ibuprofen (1.5 ng/sec) and the signal to noise ratio (S/N) is excellent for a single scan (Fig. 4a). It can be increased by spectral averaging procedure (Fig. 4b) due to the persistant ion beam allowing experiments to be conducted in the picogram range.

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This persistence of the ion beam can also be used advantageously for complex MS^n experiments. MIKES/CA spectra obtained in FF3 for the ion at m/z 161 of ibuprofen selected in the ion source (MS^2), and after dissociation in FF1 (MS^3) were compared and the spectra obtained in both experiment showed good similarity indicating that the DLI system can be helpful for that type of analysis, that are difficult to conduct easily using conventional direct insertion probes.

The simple DLI system, using a conventional direct GC/MS interface, described in this study can be very useful to interface capillary liquid chromatography and can also be used as an independent introduction system for specialized MS or MS/MS mass spectral analysis requiring persistent ion beams. The system is simple, easy to operate, offers good stability when operated under optimum conditions and is easily transportable from one instrument to another. It should easily be installed in any laboratory using a GC/MS instrument.

REFERENCE

J.P. Gagné and M.J. Bertrand. 38th ASMS Conf. on Mass Spec. All. Topics, Tucson, AZ, June 3-8, 1214-1215
The free-jet, molecular-beam mass spectrometer at SERI has been recently coupled with an Extrel triple quadrupole mass analyzer. This unique instrument (a schematic is shown in Figure 1) enables us to identify the structure of various pyrolysis products from several energy-related processes currently under study by SERI researchers. Among these projects are solar detoxification of hazardous waste, biomass characterization, zeolite upgrading of pyrolysis oils from biomass and municipal solid waste (MSW), and pyrolysis of cellulose, paper and MSW.

A brief description of the above instrument is given here. Samples are extracted through the orifice, undergoing an adiabatic, free-jet expansion to form a molecular beam. This beam is then extracted through a skimmer and a collimating orifice into the ionization region where samples are ionized via electron impact. Only positive ions are focussed and passed to the triple quadrupole mass analyzer. Collision-induced dissociation of the desired parent ion selected from the first quadrupole (Q1) takes place in the second quadrupole (Q2). Argon is used as the collision gas. The resulting fragment ions are then filtered by the third quadrupole (Q3) and detected by an electron multiplier.

Collision-induced dissociation (CID) studies of the usually complex mixtures provide important structural information about the gaseous products formed from precursor materials either by direct pyrolysis or in the presence of catalysts. They are also helpful to the understanding of mechanisms of reactions taking place in these thermal processes. For example, Figure 2 compares the CID mass spectrum for daughter ions of the m/z 106 parent that is produced in small abundance from high temperature (700°C) oxidation of chloronaphthalene, a model hazardous chemical, with that of benzaldehyde. Similar studies have positively identified the following products from this process: 1,4-naphthadione, 1,3-indandione, indenone, and their chlorine derivatives. Additional examples are identification of methyl chloride as a pyrolysis product from MSW, acetone from both MSW and wood and 2,6-dimethylphenol from poly[2,6-dimethyl-p-phenyleneoxide].

We have also applied multivariate analysis to the CID spectra to deconvolute the relative amounts of components that contribute to the same parent ions. Figures 3 to 5 show one such example. In the mass spectrum of cellulose and paper pyrolysis, an abundant peak at m/z 60 is observed. CID studies of this m/z 60 ion as well as m/z 60 ions from three pure compounds, acetic acid (HAc), hydroxyacetaldehyde (HAA) and levoglucosan (LEVO), were undertaken. The factor diagram is shown in Figure 3, in which scores of factors 2 and 3 are displayed. It indicates that this m/z 60 peak is well represented as a mixture of these three pure compounds with almost unique daughter ions at m/z 45 (HCO2*), 30 (H2CO*) and 32 (CH2O*), respectively. The relative amounts of the three components deconvoluted from factor analysis for the four paper samples tested are given in Figure 4. CID mass spectra of m/z 60 ions from the three pure compounds and a paper sample are compared in Figure 5.
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Schematic of the MB-MS/MS Sampling System

CID Mass Spectrum of m/z 106 from oxidation of 2,5-dichlorophenol

Figure 1

CID Mass Spectrum of m/z 106 from benzoic acid

Figure 2

CID of m/z 60 from Cellulose Pyrolysis
Deconvolution by Factor Analysis

Figure 3

CID of m/z 60 from Cellulose Pyrolysis

Figure 4

Figure 5

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REAL TIME DETECTION OF HF AND HCl IN AMBIENT AIR USING A MOBILE APCI/MS/MS SYSTEM

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Hydrogen fluoride (HF) and hydrogen chloride (HCl) are colourless and corrosive gases, highly irritating and toxic, even at low concentrations. Both gases are widely used in industry and are known to be byproducts of large-scale municipal waste incineration processes. HF is also used in the manufacturing of chlorofluorocarbons (CFCs), aluminum production and uranium processing. Commercial detectors lack the capabilities for sub ppb sensitivity and real time detection. Thus, a method was developed to identify and quantify ambient HF and HCl present at sub ppb levels in a complex chemical matrix.

This method involved the real time analysis of ambient air with an atmospheric pressure chemical ionization tandem mass spectrometer (APCI/MS/MS, TAGA 6000). The ion chemistry under APCI conditions is dominated by dissociative attachment reactions:

\[ R^- + XH \rightarrow X^- + HR \]

where R refers to OH, SO₂ or CO₃, and X refers to F or Cl. Other reactions observed in an APCI source lead to the formation of adduct ions:

\[ YZ^- + XH \rightarrow YZ^- \cdot HX \]

where YZ may be OH, O₂ or NO₂. The X⁻ ion may arise from a variety of F⁻ or Cl⁻-containing compounds so that F⁻ or Cl⁻ is only a good identifier of HF or HCl in the absence of other fluorinated or chlorinated species.

A background-subtracted single mass spectrum of HCl obtained by admitting trace amount of gaseous HCl to the TAGA APCI source is shown in Figure 1. The anions at masses (M-1),

![Figure 1: Single Mass Spectrum of HCl](image)

![Figure 2: Daughter Ion Spectrum of O₂⁻•HF](image)
(M+17), (M+32), (M+46) and (M+61) amu are produced by HCl deprotonation, and clustering with OH\(^-\), O\(_2\)\(^-\), NO\(_2\)\(^-\) and HCO\(_2\)\(^-\). In the tandem mass spectrometer major adduct parent ions, O\(_2\)\(^-\)-HX and OH\(^-\)-HX, are selected by the first quadrupole (Q1). These adduct ions then collide with argon gas in the second quadrupole (Q2, RF only) undergoing collision activated dissociation (CAD) to form daughter ions which are mass analyzed by the third quadrupole (Q3). Each compound has its distinct daughter ion spectrum. Figures 2 and 3 are examples of daughter ion spectra of O\(_2\)\(^-\)-HF and O\(_2\)\(^-\)-H\(^3\)Cl. These established spectra are used to identify HF or HCl in a complex matrix including other fluorinated or chlorinated compounds. The natural abundance of \(^3\)Cl and \(^37\)Cl isotopes can also be used for further confirmation of HCl.

Calibrations are performed by monitoring the real time response of the TAGA to known gas phase concentrations of the compound. The parent/daughter ion pairs, mainly O\(_2\)\(^-\)-HX/O\(_2\)\(^-\), O\(_2\)\(^-\)-HX/X\(^-\) and OH\(^-\)-HX/X\(^-\), are selected to monitor the hydrogen halides. An example of a HF calibration is shown in Figure 4: parent/daughter ion pairs of 52/32, 52/19 and 37/19 were calibrated at five different concentrations. Since HF reacts with glass and silica, the glass sampling system was silanized to lessen wall effects of the air inlet system.

The standard monitoring period for air quantity data is thirty minutes with instantaneous measurements acquired every five seconds. All monitoring data are correlated with meteorological information, which is also recorded by the mobile unit. This method had been field tested downwind of municipal waste incinerators. Real time detection limits were in the range of 0.2 to 0.5 \(\mu g/m^3\).

Reference:
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TANDEM MS ANALYSES OF ATRAZINE WITH PCI, NCI AND FAB IONIZATION

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Atrazine is one of the most widely used herbicides in the United States and has caused great environmental concern. A study of atrazine fragmentation by tandem mass spectrometric techniques may facilitate our understanding of the degradation processes of atrazine in the environment. Also, the current GC/MS method of atrazine analysis which uses EI ionization has limited sensitivity because of thermal decomposition and extensive fragmentation of atrazine under the GC/MS conditions. These problems may be avoided by using direct probe tandem MS and a softer ionization technique. Specificity is provided by using two-stage mass selection.

Tandem MS analyses of atrazine were performed on a Kratos MS-50 Triple Analyzer (EBE configuration, Manchester, UK) with PCI, NCI and FAB ionization techniques. The mass resolution of MS-I (BE) and MS-II (E) were about 3000 and 100 respectively. An Ion Tech saddle-field atom gun was used to produce 6 - 8 keV Ar for FAB ionization. CH₄ (ca. 0.1 torr in the source) was the reagent gas for Cl ionization. Glycerol and a mixture of dithiothreitol and dithioerythritol (DT) were used as matrices in the FAB experiment. The atrazine standard was purchased from AccuStandard (New Haven, CT). Helium was used as the collision gas to reduce the parent ion beam by 50%.

The high-energy CAD spectra of major ions produced in the three soft ionization methods mentioned above, show different pathways of fragmentation. Selected spectra are shown in Figure 1. For example, under the CH₄-NCI conditions used, the C-Cl bond of the atrazine radical anion, M⁻ (m/z 215), is weakened and loss of HCl is favored (see spectrum A); cleavage of C-Cl is not significant in the EI spectrum of atrazine. However, spectrum B indicates a stronger C-Cl bond in the [M - 1] anion formed under the same CH₄-NCI conditions. As reported previously (1), the H at the amino site is very active. Most likely, an elimination of H at the amino site promotes the cleavage of -CH₃ and makes the C-Cl bond less vulnerable.

The [M + 1] anion is also abundant in the NCI spectrum, but actually contains two components; one is the 37Cl isotopic ion of [M - H]⁺, and the other is [M + H]⁺. This is indicated by the two ions of m/z 179 and 181 observed in spectrum C.

The CAD spectra of the protonated molecular ion of atrazine, generated by using CH₄-PCI and FAB ionization, show an HCl loss of very low abundance at m/z 180. In general, when a halogen is attached to an aromatic nucleus, a significant signal of [M + H - Cl]⁺ ion would be anticipated. This suggests that the proton may not incorporate at the Cl site. A further study of ion m/z 180 by MS/MS/MS reveals a consecutive elimination of mass 42 (C₃H₆ and/or C₂H₄N) from m/z 180. This information regarding the fragmentation pathways of atrazine implies that the degradation processes of atrazine may be different depending on the environmental conditions.

In the FAB spectrum of atrazine in a DT matrix (Figure 2A), another abundant ion of m/z 182 is present in addition to [M + 1]. Measurement of the accurate mass of this ion has allowed an elemental composition to be assigned which corresponds formally to substitution of the Cl on the triazine ring with H, followed by protonation. Such beam induced halogen substitution reactions have been reported previously (2-4). In references 2-4 a free radical mechanism was used to account for the Cl substitution. An alternative reduction mechanism is presented in reference 4.

Experimental evidence exists which indicates that the ring stabilized free radical R₁₉₀ produced from homolytic cleavage of the C-Cl bond participates in reactions with matrix derived radical species under FAB conditions (5). For example, the ion seen at m/z 334 in a DT matrix can be accounted for by considering that the R₁₉₀ radical couples with a DT - H₋ RISS radical to produce a species with a mass of 333, which is protonated to give the ion at m/z 334. This mechanism is supported by the accurate mass and the MS/MS spectrum of m/z 334. The He CAD spectrum of m/z 182 is shown in Figure 2B.

In our preliminary FAB analyses, a nanogram quantity of atrazine provided satisfactory CAD spectra for the ions at m/z 216 and 182, indicating that a detection limit of low ppt levels of atrazine in environmental samples may be achieved by using direct probe FAB MS/MS technique. Peak profile monitoring using a DT matrix results in an acceptable signal from 20 pg of atrazine mixed with the matrix on the probe tip (5).

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Figure 1

Atrazine

(C\textsubscript{3}H\textsubscript{9})\textsubscript{2}CHNH\textsubscript{2}C\textsubscript{N}NCH\textsubscript{2}CH\textsubscript{3}

Figure 2

FAB/DTT-DTE Matrix, full scan mass spectrum

FAB/DTT-DTE Matrix, CAD mass spectrum of cation of m/z 182

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APPLICATION OF LIQUID SIMS MS/MS TO THE ANALYSIS OF SULFONATED AZO DYES

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Introduction

Sulfonated azo dyes are among the most important and widely used textile dyes. The most recent available figures indicate that each year the textile industry uses approximately 57 million pounds of acid and direct azo dyes—most of which are sulfonated compounds [1]. It has been estimated that 15% of the total dye produced is lost in the manufacturing and application process, with most of the dye being discharged in the effluent of textile mills [2]. Because recent laboratory studies have shown that both the intact azo dye molecule and the aromatic amine metabolites cause cancer in test animals [3,4], sulfonated azo dyes have become a concern of the U.S. Environmental Protection Agency. Currently, methods are being sought to better characterize and identify these azo dyes and their transformation products, in order to predict their transport and transformation in receiving waters.

Due to their involatility, sulfonated azo dyes have required techniques other than conventional EI mass spectrometry for their analysis. LC/MS [5-6] has been useful for providing molecular weight information; FAB mass spectrometry [7] and LC/MS/MS [8-9] have provided some structural information, such as ions due to the sulfonate moiety and due to cleavage at the azo linkage. In an attempt to obtain further structural information to better characterize sulfonated azo dyes, we have undertaken a systematic study of eight monosulfonated and disulfonated azo dyes using negative ion liquid SIMS-tandem mass spectrometry. In addition to obtaining the common "azo cleavage" ions [7] (formed by cleavage of the C-N bond on either side of the azo linkage and by cleavage of the N=N bond of the azo linkage) and $SO_3^-$ ions, we obtained many other structurally characteristic ions, previously unreported for sulfonated azo dyes.

Experimental

Azo dyes were supplied by the U.S. Environmental Protection Agency's Risk Reduction Engineering Laboratory, Cincinnati, OH, which had obtained the active colorants from the Ecological and Toxicological Association of Dyestuffs Manufacturers (ETAD). The colorants had been prepared synthetically and were successively precipitated to remove impurities. In each case, reverse phase high performance liquid chromatography (HPLC) showed the dyes to have only one major component. Liquid SIMS MS/MS experiments were performed on a VG 70-SEQ hybrid mass spectrometer. The cesium gun was operated at 1.2 $\mu$A and 15 kV. (M-Na)$^+$ parent ions were selected (at a resolution of 1000 and at 8 kV accelerating voltage) using the EB section of the instrument, and then were collisionally activated in the first (rf-only) quadrupole region, using argon as the collision gas. The parent ion beam was attenuated by 50%. Collision energies (in the laboratory frame of reference) were optimized for each compound, and they ranged from 110 to 150 eV. Daughter-ion scans were recorded at unit resolution using the multichannel acquisition (MCA) mode; 5 scans were accumulated at a scan rate of 6 s for the mass range 40 to 800 Da. Glycerol was used as the mass calibrant, and 3-nitrobenzyl alcohol was used as the matrix.

Results and Discussion

In addition to eliminating interfering matrix ions, liquid SIMS MS/MS produced a greater variety of fragment ions than have previously been reported for sulfonated azo dyes. The following types of ions were observed: azo cleavage ions, $SO_3^-$ and $HSO_3^-$ ions, and ions believed to be due to loss of $SO_2$, $SO_2$, $SO$, $N_2$, $CO$, and aromatic substituents $NO_2$, $HNO_2$, $NHCOCH_3$, $COCH_2$, and $COCH_3$. Figure 1 shows an example of one of the collisional activation (CA) product ion spectra obtained, and Scheme I displays the possible pathways for the formation of product ions. Many of the product ions observed were azo cleavage ions. All of these azo-type fragments that were formed directly from the (M-Na)$^+$ parent ion contained at least one sulfonate group as part of their structure; these ions are listed.
in Table 1. To date, azo cleavage fragment ions that have been observed for sulfonated azo dyes (in the negative ion mode) have contained at least one sulfonate moiety. We observed many azo cleavage product ions that did not contain sulfonate groups. These ions are listed in Table 2. Many of these ions were the result of subsequent loss of SO$_2$ or SO$_3$ from an azo cleavage ion. The fragmentation observed should aid future identifications of sulfonated azo dyes. In addition, losses of SO$_2$ were detected for all eight dyes studied, and this mass difference of 64 Da may be a useful diagnostic tool for screening sulfonated azo dyes in complex environmental mixtures.

Table 1. Azo Cleavage Product Ions Containing Sulfonate Groups

<table>
<thead>
<tr>
<th>Dye</th>
<th>Ion (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Orange 7</td>
<td>171, 156</td>
</tr>
<tr>
<td>Acid Orange 8</td>
<td>168, 170</td>
</tr>
<tr>
<td>Acid Red 1</td>
<td>381, 338</td>
</tr>
<tr>
<td>Acid Black 1</td>
<td>501, 488, 436, 366, 287</td>
</tr>
<tr>
<td>Direct Yellow 4</td>
<td>495, 494, 480, 359, 358</td>
</tr>
<tr>
<td>Direct Yellow 28</td>
<td>303</td>
</tr>
<tr>
<td>Acid Blue 113</td>
<td>451, 422, 310, 309, 298, 297, 171, 156</td>
</tr>
<tr>
<td>Direct Violet 9</td>
<td>342, 319, 318, 313, 312, 304, 206, 171, 156</td>
</tr>
</tbody>
</table>

Table 2. Azo Cleavage Product Ions Without Sulfonate Groups

<table>
<thead>
<tr>
<th>Dye</th>
<th>Ion (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Orange 7</td>
<td>167</td>
</tr>
<tr>
<td>Acid Orange 8</td>
<td>121, 106</td>
</tr>
<tr>
<td>Acid Black 1</td>
<td>333, 287</td>
</tr>
<tr>
<td>Direct Yellow 4</td>
<td>192</td>
</tr>
<tr>
<td>Acid Blue 113</td>
<td>358</td>
</tr>
<tr>
<td>Direct Violet 9</td>
<td>277, 262, 233</td>
</tr>
</tbody>
</table>

Scheme I. Proposed fragmentation for Direct Violet 9.


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EFFECTS OF IONIZATION METHOD ON COLLISION-INDUCED DISSOCIATION MASS SPECTRA OF NONIONIC SURFACTANT MATERIALS

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The methods used to form parent ions for low energy (electron-volt) collision-induced dissociation (CID) mass spectra may influence the appearance of the daughter ion mass spectra (1). This may allow additional structural information to be obtained through low energy CID. Further, one ionization method may be preferred for acquisition of structural information for a particular class of compounds. Fast atom bombardment (FAB) ionization is often chosen for mixtures of low volatility, polar compounds but FAB is not necessarily the best or only ionization method available. With appropriate introduction methods, chemical ionization (CI) or thermospray could provide alternative methods for particular compound classes.

One such class of compounds are the nonionic surfactants, ethoxylated or propoxylated ethers of long-chain aliphatic alcohols or alkyl-substituted phenols. Other, more polar, functionalized molecules have also been employed as surfactant hydrophobes. These commercially important materials are produced and used as complex mixtures, characterization of which requires information on number of components present, length and composition of hydrophobe, and type and extent of alkoxylation. Low-energy CID following direct supercritical fluid injection and positive CI (SFI/CI) indicated different types of daughter ions are produced from different surfactant types (2). Diagnostic fragment ions were produced and structural information was available for some hydrophobe types.

In the current work, tandem quadrupole CID/MS/MS spectra were produced following FAB, thermospray and desorption chemical ionization (DCI). Results were combined with those from the SFI/CI study and compared to determine the extent to which ionization mechanism influences the type and amount of fragment ions produced. A single mass spectrometer with variable ion sources was employed. Six different types of commercial nonionic surfactant mixtures were analyzed in an effort to produce ions diagnostic of hydrophobe structure. Information to determine the mechanisms of ion decomposition was also sought. Results were to be used to define the most appropriate combination of introduction and ionization techniques for rapid structural characterization of nonionic surfactant mixtures.

Results indicated qualitative differences are apparent in the daughter ion spectra produced. These differences are noted without regard to the type of nonionic surfactant mixture studied. The use of different ionization methods does not appear to lead to different types of ions for the surfactants studied but to the increase of one type of fragment ion over other ions in the spectrum.

Fast atom bombardment (FAB) ionization appears to be the most energetic mechanism compared with SFI/CI, DCI or thermospray. FAB ionization prior to CID leads to more abundant fragment ions at lower collision energies than for the other methods. This may be due, in part, to the lack of collisional cooling in the lower pressure FAB source, as compared with the CI or thermospray sources. This higher energy ionization may also be responsible for the difficulty in producing protonated molecules for the nonionic surfactants used in this study. The addition of
alkali metal salts results in production of more abundant parent species in FAB, but these are more difficult to dissociate under low energy CID conditions (3,4).

Chemical ionization following supercritical fluid injection appears slightly more energetic than either DCI or thermospray. Reasons for this are not readily apparent, but the differences appear very slight. Thermospray ionization appears to be the least energetic approach of those studied but again only by a small amount. This may be influenced by the production of ammonium adduct ions in thermospray, with some energy in CID dissipated through loss of the solvated ammonia molecule. Future efforts will address production and CID analysis of protonated molecules in thermospray.

Although FAB is more energetic than other approaches, it still does not allow complete hydrophobe characterization of all nonionic surfactant types. Difficulty in producing ions for CID is one limitation, along with the apparent energy limitation of the tandem quadrupole system. Other approaches using supercritical fluid injection/CI may be useful, such as charge-exchange (CE) ionization and CID of CE-produced fragment ions. Further work is underway to address these issues.


Proposed ether-cleavage mechanism to explain formation of protonated poly(ethylene glycol) ions and loss of hydrophobe as an alkene.
TANDEM QUADRUPOLE CID OF ORGANOMETALLIC IONS PRODUCED DURING FAB STUDIES OF PALLADIUM-MEDIATED C-NUCLEOSIDE SYNTHESIS REACTIONS

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Carbon-carbon bond formation in organic synthesis can be accomplished through use of palladium (II) salts in a mechanism, known as the Heck reaction (1), involving a reactive arylpalladium species and a carbon-carbon double bond. A version of this reaction, with the C-C double bond present in a cyclic enol-ether or glycal, has been extensively studied as a route for C-nucleoside synthesis (2). This route is believed to involve four distinct organometallic reaction steps: (a) transmetalation from an arylmercury salt to form the organopalladium reagent (R-Pd), (b) π-complex formation between the palladium and glycal double bond, (c) π-complex collapse and formation of a σ-adduct, and (d) σ-adduct decomposition to products. Isolation and characterization of each of the organopalladium intermediates of this reaction has been difficult due to their highly reactive nature.

Fast atom bombardment (FAB) ionization provides a means to study species in liquid solution using mass spectrometry and has been used to study solutions of organopalladium reagents (3,4). The study of C-nucleoside synthesis via organopalladium reactants using FAB/MS (3) gave strong evidence to verify the structure of the reactive arylpalladium intermediate, R-Pd. There was some ambiguity, however, regarding the structures of some of the metal-containing ions produced in that study. The relationships between the solution chemistry of the species and gas-phase ions and between ions and reaction products was not directly apparent.

The present work extends the previous effort (3) by studying some of the FAB-produced ions using tandem quadrupole mass spectrometry. The aim was to produce structurally significant fragment ions to ascertain details of the metal-glycal bonding in the reactive intermediates. Ions produced in collision-induced dissociation (CID) may also reveal details of the decomposition mechanisms of metal-containing intermediates to known reaction products.

Tandem quadrupole mass spectrometry, in conjunction with fast atom bombardment (FAB) ionization, increases the amount of information available for
reactive organometallic reagents in solution. Collision-induced dissociation in the low energy (electron-volt) regime is capable of producing metal-containing fragment ions characteristic of structures of organometallic reaction intermediates. Preliminary identification of some of these fragment ions supports the proposed structures of selected reactive intermediates in the palladium-mediated synthesis of C-nucleosides.

The results of the FAB/MS/MS study of the palladium-mediated reaction of pyrimidinedionyl mercury with triacetylglucal indicate that conditions used in the current study do not completely correspond with conditions used in a previous, more limited effort (3). Many of the same intermediate and product-related ions found in the earlier work were detected in the present study. The extent of fragmentation, for both organic and organometallic ions, in the single quadrupole FAB/MS in the current work suggest that FAB mass spectrometry conditions were more energetic than used in the previous study. The high energy present in the FAB/MS appears to favor the production of certain products from this reaction.

Although not completely clear from the data, the analytical conditions appear to favor the detection of one form of the principal organometallic intermediate over the other possibility. The more stable σ-bonded adduct appears responsible for the m/z 517 glycal-palladium-pyrimidinedione ion. An ion corresponding to the reactive pyrimidinedione-palladium reagent (PyPd, m/z 245) was not found. This condition complicates the analysis of the reaction mixtures, as it is not clear to what extent the analytical method or conditions dictate which ions will be formed.

Further work is underway to more fully duplicate the analytical conditions used in the previous work. It remains to be determined if and how the design and operation of the tandem quadrupole mass spectrometer may be directing the preference for one bonding form of the organometallic intermediates. Other reactions and analytical conditions are also being explored.

The 39th ASMS Conference on Mass Spectrometry and Allied Topics

EPIMER DISTINCTION AND STRUCTURAL EFFECTS ON GAS-PHASE ACIDITIES OF ALCOHOLS MEASURED USING THE KINETIC METHOD

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The kinetic method is used to estimate gas-phase acidities ($\Delta G^{\circ}_{\text{acid}}$) of two sets of epimeric methylcyclohexanols. Metastable decomposition of proton-bound dimers of alcohols formed by negative chemical ionization in a Finnigan TSQ 4500 mass spectrometer is used. MS/MS product spectra of the proton-bound dimers of epimers of 4-methylcyclohexanol and 3-pentanol (Fig. 1) clearly show differences in gas-phase acidities of the epimers. Structures and $\Delta G^{\circ}_{\text{acid}}$ values of the four isomers a-d of methylcyclohexanol as calculated using a calibration curve (Fig. 2) are shown in Scheme 1. Comparing these $\Delta G^{\circ}_{\text{acid}}$ values for the two sets of epimers a vs. b and c vs. d, it is clear that in both cases the cis-isomers have lower gas-phase acidity values than their corresponding trans-isomers as a result of destabilization of the alkoxide anions due to 1,3 diaxial interactions between the axial hydrogens and the oxygen atom. Location of the methyl group in the 2-position of the cyclohexanol ring lowers the $\Delta G^{\circ}_{\text{acid}}$ values due to destabilization of the alkoxide anion by gauche interactions of the 2-methyl group.

![Figure 1: MS/MS product spectra of the proton-bound dimers of 3-pentanol and cis-4-methylcyclohexanol (a) and trans-4-methylcyclohexanol (b).](image)

![Scheme 1.](image)
Effective temperatures for metastable dissociation of proton-bound dimers of various alcohols are calculated from the slopes of the calibration curves and summarized in Table 1. It is evident from Table 1 that for homogenous proton-bound dimers, alkyl substitution at the carbon atom having the hydroxyl-group has a great influence on the effective temperature. The extent of change depends on the number of substituted alkyl groups; the largest effect being observed during the first alkyl substitution and the effect decreases for second and third substitutions. For the heterogenous proton-bound dimer systems, dissociation of primary alkoxide anions proton-bound to secondary alkoxide anions shows the largest effective temperature of 550K whereas secondary/tertiary systems show an effective temperature of 340K (Table 1). An increase in effective temperature is also observed when the experiment is done under CID condition compared to that observed under metastable condition. Similar results were reported for experiments performed at different target (argon) pressures and collision energy. The increase in effective temperature is due to the increase in internal energy of the ion as a result of increasing collision pressure or collision energy. The highest temperature in Table 1 is shown by the proton-bound dimers of the mixed system, primary and secondary alcohol. This is a case where two different functional groups are attached to a proton by weak hydrogen-bonds. In such a situation the frequencies of vibration of the hydrogen bonds in the proton-bound dimer will be different. As a result the effective temperature needs to be corrected for frequency factors. Similar results were reported in case of formate/acetate systems where the alpha-carbon atoms were different and difference in slopes of the calibration curves were observed.

Table 1. Effective temperatures $T$ calculated for proton-bound dimer systems of different alcohols.

<table>
<thead>
<tr>
<th>Proton-bound alkoxide ions</th>
<th>$T$ (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Primary) $\text{H}^+.(\text{Primary})$</td>
<td>460</td>
</tr>
<tr>
<td>(Secondary)$\text{H}^+.(\text{Secondary})$</td>
<td>290</td>
</tr>
<tr>
<td>(Tertiary)$\text{H}^+.(\text{Tertiary})$</td>
<td>360</td>
</tr>
<tr>
<td>(Primary)$\text{H}^+.(\text{Secondary})$</td>
<td>550</td>
</tr>
<tr>
<td>(Secondary)$\text{H}^+.(\text{Tertiary})$</td>
<td>340</td>
</tr>
</tbody>
</table>

Figure 2: Logarithm of relative rates of dissociation of proton-bound dimers of a series of secondary alcohols (using 3-pentanol as reference) vs. gas-phase acidity.

References

THE CHARACTERISATION OF SUBSTITUTED TRIPHENYLENE COMPOUNDS
BY FIELD DESORPTION - MASS SPECTROSCOPY

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INTRODUCTION

Field Desorption - Mass Spectrometry (FD-MS) is used in our laboratory as a lead characterisation tool in the analysis of complex mixtures of industrial significance. We have recently studied a selection of substituted triphenylene compounds of structure:

where R may be either -O,CPhOC,H_{2n+1} (Ph = 1,4-Substituted phenyl group) or -OC,H_{2n+1}. These materials have been analysed using both FD-MS and FD-MS-MS methodology and this combination of techniques has provided information unobtainable other techniques.

RESULTS AND DISCUSSION

(a) Triphenylene Esters

An example of the type of FD-MS spectrum obtained for this compound class is shown below for the C_{20} ester.

The fragment at m/z 401 represents cleavage at the carbonyl bond and provides good diagnostic information regarding the alkyl chain feedstock used in the synthesis of these materials. This extent of fragmentation is somewhat unusual in FD data and appears not to be as might be expected, thermal in nature but occurs as a result of a combination of complex field-induced mechanisms.

(b) Triphenylene Ethers

The second class of triphenylene compounds included in this study were ethers of alkyl chain length C_{8} to C_{12}. The type of FD-MS spectrum obtained for this compound class (for the C_{8} ether) is shown below.
It is immediately interesting to note the lack of fragmentation observed in this spectrum when compared to the ester data. Also of interest is to note the occurrence of peaks corresponding not only to the expected molecular ion (at m/z 744) but also a peak at m/z 814 which is consistent with the presence of an additional alkyl group in the molecule. The origin of this 'additional' peak is further discussed in the tandem mass spectrometry section of this poster.

(c) FD-MS-MS

FD spectra are generally dominated by molecular ion species and as such are ideally suited for analysis by tandem mass spectrometric methods.

One of the unresolved issues connected with the analysis of the triphenylene ether compounds concerned the nature of the peaks occurring at masses greater than the expected molecular weight and FD-MS-MS has been used to further investigate this issue. The spectrum shown below is the product ion spectrum obtained by subjecting the C₅ molecular ion (m/z 744) to high energy (4kV) CID using He as the collision gas.

The m/z 744 product ion spectrum demonstrates losses of side chain moieties together with associated losses of oxygenated side chain moieties for the more intense product ions. This still leaves unresolved the nature of the extra alkyl substitution occurring at m/z 814 for the C₅ ether. The product ion spectrum obtained for this ion is characterised by a high yield of product ions consistent with the intact, fully substituted C₅ triphenylene ether. This indicates that the m/z 814 product is related to the fully substituted ether molecule and is not an experimental artefact.

ACKNOWLEDGEMENT

The authors thank R Vickers, VG Analytical Ltd for his assistance in obtaining the high energy CID MS-MS data.
TANDEM MASS SPECTROMETRIC STUDIES OF UNUSUAL EI FRAGMENTATION PATHWAYS OF 5-NITROOCTAETHYLPORPHYRIN AND ITS DIVALENT METAL COMPLEXES

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INTRODUCTION

Porphyrins chelate with virtually all metallic elements. The chelated ion can have a strong influence on the spectroscopic properties of the complex.

In the EIMS analysis of octaethylporphyrin (OEP) and its metal complexes, $\beta$-cleavage of the peripheral ethyl groups is the predominant mode of fragmentation. In studies performed by Beato et al., it was determined that insertion of a metal into the free-base OEP markedly influenced $\beta$-cleavage intensities. The influence of the metal was found to be important in determining the abundance of ions in the doubly charged region of the mass spectrum, whereas the singly charged region was relatively unaffected.

In our studies, the insertion of metals into 5-nitro-OEP was found to have a strong influence on the types of fragmentation encountered in the singly charged region of the mass spectrum. The divalent metal complexes studied include Co, Cu, Zn, Ni, Pd, Ag and Mg. The effect of the metals on the fragmentation pathways were significantly different for each of the metals studied.

Clezy et al. determined that the presence of a nitro group at one of the bridge carbons modified the normally encountered beta-cleavage of OEP. In our MS/MS studies of free-base 5-nitro-OEP, daughter spectra showed a significant $[M-17]^+$ peak due to loss of OH$^-$ as was reported by Clezy in his EIMS work. We have also found evidence for a novel ring fragmentation of 5-nitro-OEP not observed for OEP under EI conditions.

EXPERIMENTAL

All mass spectra presented here were obtained with a Finnigan MAT TSQ45 triple quadrupole mass spectrometer. Porphyrin samples were dissolved in dichloromethane and 1 $\mu$L aliquots of the solution were deposited onto a direct exposure probe (DEP) filament. The DEP was heated from 100 to 600 $^\circ$C at $600^\circ$C/min. The rapid rate of heating was found to reduce the amount of thermal elimination of NO$_2$ observed in EIMS.

The spectra were obtained under EI conditions. The filament emission current was maintained at 0.3 mA and the electron energy was 70 eV for all experiments. For the initial EIMS experiments quadrupole 1 (Q1) was scanned from 50 to 800 m/z in 0.8 s with Q2 and Q3 passing all masses. Tuning for MS/MS was achieved with Co(II) OEP in a capped aluminum solids probe vial heated to 275$^\circ$C. The MS/MS experiments were performed with a collision gas pressure of 1.6 mTorr argon and a collision energy of 24.8 eV.

A Finnigan MAT 95 high resolution mass spectrometer was used for determination of exact masses for fragment ions. The MAT 95 was tuned for a resolution of 7000. The high resolution data were used as an aid in determining the composition of certain fragment ions.

5-nitro-OEP was chosen for these studies because its spectroscopic properties have been extensively studied. Porphyrin compounds were synthesized according to literature procedures.

RESULTS

The fragmentation exhibited by 5-nitro-OEP was found to be different from the fragmentation of OEP. We have found that pyrrole ring cleavage occurs for 5-nitro-OEP under EI conditions. The daughter spectrum of $M^+$ is shown in Figure 1. The composition of the m/z 375 ion was confirmed by high resolution mass spectral data and is shown in Figure 2. Metallation of 5-nitro-OEP altered the fragmentation exhibited. Pyrrole ring cleavage was unobserved, but $[M-75]^+$ or $[M-86]^+$ ions appeared in daughter spectra of $M^+$ for all of the metal complexes studied. The
fragments associated with both of those ions are shown in Figure 3. Parent spectra for the [M-75]+ and [M-86]+ ions revealed that the fragmentation for both ions was concerted within the timescale of collision-induced dissociation.

Figure 4 displays a trend in the intensity of the [M-86]+ peak when plotted versus the number of d electrons of the inserted metal. The general trend is paralleled by the dependence of the metal-sensitive IR bands of OEP to the d electrons of the inserted metal.

CONCLUSIONS

The fragmentation of 5-nitro-OEP was found to be different from that of OEP. We have found evidence for pyrrole ring cleavage in 5-nitro-OEP. Oxygen migration seems to be an important part of the process. With metallation of 5-nitro-OEP, the modes of fragmentation are significantly altered. A peak at either [M-75]+ or [M-86]+ was the base peak in the daughter spectra of the M+ ions of all of the metallated species. The intensity of the [M-86]+ ion was found to increase as the number of d electrons of the inserted metal decreased. Similar trends were previously found for the metal-sensitive IR bands of OEP.

INTRODUCTION: Many unusual fragmentation and rearrangement reactions are observed in the mass spectra of organosilicon compounds. These reactions can make structural elucidation of some relatively simple organosilanes difficult based solely on EI MS data. A collisionally activated dissociation (CAD)/MS study was conducted to provide more information on the mass spectrometry of tetraethoxysilane (TEOS) and its carbon analog, tetraethoxymethane (TEOM). Utilizing the fragmentation mechanisms elucidated by this CAD study, an empirical system for the identification of unknown alkylalkoxysilanes in complex mixtures was developed.

EXPERIMENTAL: Electron impact (EI) mass spectra were acquired on a VG Trio 3 triple quadrupole mass spectrometer. Samples were ionized at 70 eV ionization energy with a source temperature of 200°C. Constant sample pressure was provided by injection of 1 to 2 uL of sample into the heated septum inlet. Argon was used as the collision gas for all CAD experiments. The pressure in the collision cell was varied from 0.05 to 2 mTorr, with 0.2 mTorr being optimal. Typical collision energies ranged from 0.1 to 20 eV (laboratory frame). GC/MS spectra were acquired on a VG 7070H sector mass spectrometer. All separations were made on a DB-5 0.25 mm ID column temperature programmed from 50 to 300°C.

RESULTS AND DISCUSSION: The EI spectrum of TEOS was extremely complicated compared to its carbon analog, TEOM (Figure 1). The fragmentation mechanisms of TEOS were elucidated using CAD experiments. Product and precursor scans indicated the ions; m/z 207 ([M-H]+), m/z 193 ([M-CH3]+) and m/z 179 ([M-C2H5]+) were precursors for three separate fragmentation pathways. The first fragmentation pathway was initiated by the loss of a hydrogen radical from the molecular ion to produce m/z 207 [M-H]+. The product ion spectrum of m/z 207 revealed a loss of 44 u to yield m/z 163 ([C2H5O)3Si]+ . Product scans of m/z 163 produced a series of ions resulting from consecutive losses of 28 u and 44u, consistent with the neutral losses of ethylene and acetaldehyde, respectively. The second pathway proceeded by the loss of a methyl radical from the molecular ion to produce m/z 193 ([C2H5O)3SiOCH2]+ . Product scans of m/z 193 indicated a neutral loss of 44 u (acetaldehyde) to m/z 149 (the base peak in the EI spectrum). There were no indications for the neutral loss of ethylene or the loss of -OCH₂ in this pathway. The third pathway was initiated by the loss of an ethyl radical from the molecular ion to produce m/z 179. Product ion scans of m/z 179 revealed two product ions, m/z 151 and 135 again proceeding via the elimination of ethylene and acetaldehyde, respectively. The carbon analog, TEOM, produced a simpler EI spectrum under identical MS conditions. No molecular ion was observed. Product scans of m/z 147 [M-45]+ indicated the ions m/z 119, 91, and 63 resulted from consecutive neutral losses of ethylene. The differences in complexity between the EI spectra of TEOS and TEOM were determined to result from the lack of an energetically favored pathway for the elimination of acetaldehyde from TEOM during fragmentation.
Based on the information obtained from the CAD study of TEOS, an empirical system was developed to identify unknown silanes resulting from the reactions of TEOS and metal alkyls. The compounds studied had a general formula:

$$(\text{EtO})_x\text{Si}(\text{R})_{4-x}$$

where R = ethyl, butyl, and chlorine.

The system is based on the presence or absence of three ions in the El spectrum of the unknown silane. The three ions of interest are $m/z$ 63 [H-Si-(OH)$_2$]$^+$, $m/z$ 75 [(H)$_2$Si-OEt]$^+$, and $m/z$ 79 [Si(OH)$_3$]$^+$. General formulas for the unknown silanes can be obtained by the following system:

<table>
<thead>
<tr>
<th>Present</th>
<th>Absent</th>
<th>Gen. Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m/z$ 75</td>
<td>(no m/z 63 or 79)</td>
<td>EtO-Si-(R)$_3$</td>
</tr>
<tr>
<td>$m/z$ 63 and 75</td>
<td>(no m/z 79)</td>
<td>(EtO)$_2$Si-(R)$_2$</td>
</tr>
<tr>
<td>$m/z$ 63 and 79</td>
<td>(no m/z 75)</td>
<td>(EtO)$_3$Si-R</td>
</tr>
</tbody>
</table>

After assignment of a general formula, the complete structure of the unknown silane can be elucidated by identifying ions resulting from neutral losses of alkenes.

Current research involves extending this system to larger, more complex alkylalkoxysilanes and mixed tetraalkoxysilanes.

![Figure 1. Comparison of the El spectra of (a) tetraethoxysilane (TEOS) and (b) tetraethoxymethane.](image)

References
Structural Analysis of Glycosphingolipids by Charge Exchange DCI / MS / MS
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In the analysis of permethylated glycosphingolipids with desorption chemical ionization (DCI) mass spectrometry, carbon dioxide/methanol has been reported to be a superior reagent gas than the most popular ammonia. In addition to the molecular ion and sugar sequence related fragment ions, fragment ion (F fragment) of high abundance resulting from the loss of long chain base were also observed[1]. These F fragment ions make it easy to establish the ceramide composition.

F fragment ion (or an β - cleavage ion) was first reported in the analysis of permethylated glycosphingolipids with DEI[2]. In the carbon dioxide/ methanol DCI system, it is believed that methanol was responsible for the production of protonated molecular ion whereas charge exchange between the sample molecule and the carbon dioxide radical cation was responsible for the formation of F fragment ions. In this report, charge exchange DCI with reagent gases of different recombination energy, alone and in combination with tandem mass spectrometry, were evaluated as a means of improving structural information of the glycosphingolipids.

Strong F fragment ion signals were recorded when helium, argon, nitrogen or carbon dioxide was used as the charge exchange reagent gas. These F fragment ions were either absent or present as very weak signal when ammonia or methane was used as the reagent gas. A typical charge exchange DCI mass spectrum of permethylated glycosphingolipids is shown in Fig. 1. The ion at m/z 734 is the F fragment ion. The protonated molecular ion observed in the charge exchange mass spectrum is probably due to the trace of water in the system.

Due to its low E\textsubscript{lab} value, triple quadrupole mass spectrometry is generally considered as a low energy CID instrument. Recently, several groups reported its limited CID capability for parent ion with mass to charge ratio above 800 amu. The daughter ion mass spectrum of permethylated glycosphingolipids is shown in Fig. 2. It is surprising to see that parent ion with mass to charge ratio of 1573 can be fragmented. These daughter ions are very useful in the assignment of the glycan sequence.

Although parent ion with mass to charge value over 1500 amu does fragment in the triple quadrupole mass spectrometry, unlike the high energy CID results reported by Costello[3], no ion related to ceramide composition was observed. Therefore, Ceramides ions, instead of molecular ions, were chosen as the precursor ion in the daughter ion analysis. Fig. 3 shows the daughter ion mass spectrum of ceramide ion with m/z value 578 from the permethylated N-stearoyldihydrolactocerebroside. Two major fragmentation pathways were observed. The signals at m/z 312, m/z 280 represented the long chain base ion, and the loss of methanol from the long chain base respectively. Thus, the sphingoid moiety can be assigned as dihydrospingosine. The signal at m/z 298 is the fatty acid fragment ion representing a palmitamide residue. It is interesting to see that only long chain base ions were observed (Fig. 4) when the long chain base is a sphingosine or a eicosasphingosine residue. The fragmentation mechanism which might be related to the double bond in the long chain base is currently under Investigation.

REFERENCES
Fig. 1 CO2-DCI / MS Spectrum of permethylated N-palmitoyldihydrolactocerebroside.

Fig. 2 N2-DCI / MS / MS daughter ion spectrum of F fragment ion m/z 1573 of permethylated GM1

Fig. 3 CO2-DCI / MS / MS daughter ion spectrum of ceramide ion m/z 578 of permethylated N-stearoyldihydrolactocerebroside.

Fig. 4 CO2-DCI / MS / MS daughter ion spectrum of ceramide ion m/z 576 of permethylated N-stearoyllactocerebroside.
Collision Induced Dissociation of Group IIIA Dimethyl Cations via Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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The goal of metalorganic chemical vapor deposition (MOCVD) is to deposit a metal atom to a surface without contaminating the deposition surface. This technique has become a leading contender in the fabrication of sophisticated heterojunction devices. The reactions that take the metal from the gas phase metal-organic compound to the bare metal play an important role in determining the impurities found within the materials produced by this method. These reactions are also important in determining the deposition rates of the semiconductor materials. An understanding of the ion chemistry of these compounds may also open a new avenue to getting the metal to the deposition surface in a reproducible way, while being able to monitor and reduce the amount of contamination.

Various methods have been investigated with the goal of improving the deposition of these semiconductor materials on various types of surfaces. The traditional method of thermal decomposition of the precursors has given way to plasma stimulated MOCVD, electron cyclotron resonance plasma excited MOCVD, and laser-induced photochemistry in the MOCVD process. These new methods open up the very real possibility of ion/molecule chemistry becoming directly involved in the MOCVD process and therefore influencing the purity and the characteristics of the deposition surface and the rates at which these surfaces are deposited.

The experiments were performed with a Fourier transform ion cyclotron mass spectrometer (FT-MS). The experimental apparatus consists of an Extrel FTMS-1000 data system, a 3 Tesla superconducting magnet and a vacuum system with a 1 x 1 x 2 (z-axis) inch cell made of stainless steel plates, except for the trapping plates which are composed of 90% transparent nickel mesh. The metal alkyls (M(CH$_3$)$_2$) were maintained at static pressures ranging from $0.5 \text{ to } 2.0 \times 10^{-4}$ torr and ionized via electron impact (EI). Molecular ions were observed for these compounds with increasing difficulty as one goes down the group from aluminum to indium. The reactant gases were added to give total pressures of between $3.0 \times 10^{-8}$ to $1.1 \times 10^{-7}$ torr. The reaction gases (O$_2$, H$_2$, NH$_3$) were used without further purification the H$_2$O and D$_2$O samples were freeze-pumped-thawed several times. The purities of these compounds were verified using EI.

The electron impact experiments were carried out on the TMM neutrals by varying the beam voltage to increase the internal energy of the ionized species (DMM$^+$) to induce further fragmentation. The collision-induced dissociation experiments were carried out by setting a low amplitude single frequency pulse on the desired species for a variable length of time (0.06 to 3.0 msec). This translationally excites the cation of interest which then undergoes collisions with argon.

The TMI fragmented upon electron impact to give the DMI cation. No products were observed for reactions with water but the presence of a small amount of the adduct was detected for reactions with ammonia. The EI and CID experiments with DMI$^+$ gave similar results. In$^+$ was the major product at all energies. A small amount of the TMG molecular cation was observed. The TMG cation yielded DMG$^+$ under both EI and CID conditions. No products were observed for reactions with water. An adduct was observed for reactions with NH$_3$:

$$(\text{CH}_3)_2\text{Ga}^+ + \text{NH}_3 \rightarrow (\text{CH}_3)_2\text{GaNH}_3^+$$

The CID experiments with DMG$^+$ gave Ga$^+$ and CH$_3$Ga$^+$. The ratio of the fragments did vary with energy, giving a ratio of approximately 3:1 at the high-energy limit of the CID experiment. Using EI (variable beam voltage) on the TMG neutral to produce DMG$^+$ with excess internal energy yielded no fragments.

A substantial amount of TMA molecular cation was produced. The CID of TMA$^+$ yielded DMA$^+$, MMA$^+$, and Al$^+$ with a ratio of 10:1:1, respectively. The fragmentation pattern of DMA$^+$ did depend on the internal energy of the cation. DMA$^+$ collisional activation gave Al$^+$ and MMA$^+$ with ratio of 5:1, respectively. The reaction of DMA$^+$ with ammonia yielded a mono and a di adduct. At short reaction times DMA-NH$_3^+$ was observed which reacted at longer times to produce DMA-(NH$_3$)$_2^+$ as shown in Figure 1.
DMA undergoes a sequential substitution of methyl by OH as shown in Figure 2. This reaction most likely proceeds by:

$$\text{(CH}_3\text{)}_2\text{Al}^+ + \text{H}_2\text{O} \rightarrow \rightarrow \text{(CH}_3\text{)}\text{AlOH}^+ (m/z=59) + \text{CH}_4$$

$$\text{(CH}_3\text{)}\text{AlOH}^+ + \text{H}_2\text{O} \rightarrow \rightarrow \text{Al(OH)}_2^+ (m/z=61) + \text{CH}_4$$

Masses 59 and 61 underwent CID yielding A1OH* (m/z=44) and Al*. These results were verified by using D$_2$O which gave products at mass 60 and 63. Again these products underwent CID and yielded AlOD* (m/z=45) and Al* as fragments. Many adducts were produced when TMA/DMA cations were allowed to react with H$_2$O. These products progress to about m/z equal to 300. These products grow in as the reaction time is increased with the higher mass products growing in at longer reaction times. Peaks in a series are separated by two mass units: CH$_3$ being substituted by OH. The series seem to have a separation of 18 mass units: addition of H$_2$O. The series of peaks can also be seen as being separated by DMA units. Some of the more abundant products have been studied by CID. These experiments yielded a variety of products such as DMA*, Al*, as well as losses of (CH$_3$)$_x$ with x equal to 1-3.

The three members of Group IIIA TMM and DMM cations investigated in this work exhibited different CID behavior as well as different reactivities. The fragmentation pattern of the DMM cations was shown to vary with internal energy. This is true for DMA and DMG, but DMI essentially showed no energy dependence fragmenting directly to give the metal cation which indicates that DMI* fragments exclusively via ethane elimination. Ethane elimination may also occur in DMG* and DMA*, but to only a minor extent. None of the TMMs (neutral or ionic) or the DMM cations studied reacted with oxygen or with hydrogen, a commonly used carrier gas in MOCVD. The TMMs were found to adsorb readily to the stainless steel walls of the vacuum chamber. They were also found to desorb intact from the stainless steel. Both DMG* and DMA* readily formed adducts with ammonia. DMG* produced a mono adduct and DMA* produced both a mono and a di adduct. The observation of these adducts in the experiments lends weight (not mass) to the belief that adduct formation occurs in MOCVD reactors and that these species can be a cause of the impurities observed in semiconductor materials. Direct evidence for this type of adduct formation has recently been reported by Piccos and Ault for the formation of a neutral trimethylgallium:arsine adduct.

Oxygen incorporation into TMA-produced semiconductors is most likely due to residual water in the deposition lines and not due to residual oxygen.

Addition of Ammonia to Dimethylaluminum

$$P = 3 \times 10^{-8} \text{ Torr (Ammonia)}$$

Sequential Substitution (OH for Methyl)

$$P = 2 \times 10^{-5} \text{ Torr (Water)}$$

Figure 1) Ammonia and DMA*

Figure 2) Water and DMA*

1) NRL/NRC Postdoctoral Research Associate
ENERGIES OF TRIPLET ELECTRONIC STATES OF THE \textit{N}_2\textit{O}^{2+} ION

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INTRODUCTION
The energies of electronic states of \textit{N}_2\textit{O}^{2+} have previously been measured by Auger-electron (AE) spectroscopy [1], double-charge-transfer (DCT) spectroscopy [2,3] and photolon-photolon coincidence (PIPICO) spectrometry [3,4]. In addition, these energies have been calculated [1,3,5] by several procedures. The AE spectroscopy and DCT spectroscopy (in which \textit{H}^+ was the projectile ion) experiments [1-3] gave information on energies of \textit{singlet} electronic states of \textit{N}_2\textit{O}^{2+}. The PIPICO experiment [3], however, allowed the energy of the lowest triplet state to be determined; this energy was particularly important since it was taken as the reference point for the computed energies [3].

The purpose of the present investigation was to obtain information on \textit{triplet}-state energies of \textit{N}_2\textit{O}^{2+} using a modified form of DCT spectroscopy. A high-velocity, singly charged positive ion is converted in DCT spectroscopy to a negative ion in a double-electron-capture (DEC) reaction with the molecule under investigation. There is now considerable evidence [6] that the total spin of reacting and resulting particles is conserved in such reactions. For a closed-shell species such as \textit{N}_2\textit{O}, the molecule is in a singlet state. Using a ground-state singlet projectile ion such as \textit{H}^+ gives rise to reactions which populate singlet states of the dication, since \textit{H}^+ ions which reach the detector must be in the relatively long-lived \textit{\Sigma}_0^+ singlet state. However, using ground-state triplet projectile ions, such as \textit{OH}^+ and \textit{F}^+, leads to the population of triplet states of the dication since \textit{OH}^+ and \textit{F}^+ have only singlet bound states. Thus, the present DCT spectroscopy study of \textit{N}_2\textit{O} was carried out with \textit{OH}^+ and \textit{F}^+ projectile ions in order to determine double-ionization energies to triplet states of \textit{N}_2\textit{O}^{2+}. In addition, the double-ionization energies to ground triplet and singlet states of the dication have been calculated using Møller-Plesset perturbation theory with split-valence basis sets.

EXPERIMENTAL PROCEDURE
The experiments were carried out on a VG ZAB-E spectrometer. The \textit{OH}^+ and \textit{F}^+ ions were generated by dissociative electron ionization of \textit{H}_2\textit{O} and \textit{CF}_4, respectively, and were accelerated to either 6 or 8 keV. After mass selection, they interacted with \textit{N}_2\textit{O} molecules contained in the second field-free region collision-gas cell. \textit{OH}^- or \textit{F}^- ions were transmitted to the detector by re-arranging the polarities of the voltages normally applied to the plates of the electric sector. DCT spectra were obtained by scanning these voltages.

If the projectile ion is denoted by \textit{A}^+, the DEC reaction can be represented by

\[ \textit{A}^+ + \textit{N}_2\textit{O} \rightarrow \textit{A}^- + \textit{N}_2\textit{O}^{2+} \quad \ldots(1) \]

Since the energy, \( I_2(\textit{N}_2\textit{O}) \), required to doubly ionize \textit{N}_2\textit{O} is greater than the energy \( E(\textit{A}^+ \rightarrow \textit{A}^-) \) made available from charge inversion of \textit{A}^+, the energy to drive reaction (1) must come from the translational energy \( E_0 \) of \textit{A}^+.

If the translational energy of \textit{A}^+ is \( E_1 \), then

\[ E_0 - E_1 = I_2(\textit{N}_2\textit{O}) - E(\textit{A}^+ \rightarrow \textit{A}^-) \quad \ldots(2) \]

If various states of \textit{N}_2\textit{O}^{2+} are populated, various values of \( E_1 \) result, each corresponding to a peak in the DCT spectrum.

The energy-loss scale was calibrated by carrying out a DCT spectroscopy experiment with argon atoms in the cell. If the translational energy of the \textit{A}^- ions formed in DEC reactions with \textit{Ar} is \( E_{\textit{A}^-} \), then

\[ E_0 - E_{\textit{A}^-} = I_2(\textit{N}_2\textit{O}) - E(\textit{A}^+ \rightarrow \textit{A}^-) \quad \ldots(3) \]

and

\[ E_{\textit{A}^-} - E_1 = I_2(\textit{N}_2\textit{O}) - I_2(\textit{Ar}) \quad \ldots(4) \]

In practice, \( E_{\textit{A}^-} \) and \( E_1 \) are measured and \( I_2(\textit{N}_2\textit{O}) \) is calculated using Eq. (4) since \( I_2(\textit{Ar}) \) is known.

THEORETICAL PROCEDURE
The double-ionization energies calculated in the present investigation are the differences between the total energy of the ground state of the singlet \textit{N}_2\textit{O} molecule and those of the singlet and triplet...
dication. They were calculated using the GAUSSIAN86 ab initio programme package. The geometry used for the dication was the equilibrium geometry of neutral N$_2$O. This is in keeping with the assumption that no significant geometry change occurs during the DEC reaction as the high-velocity projectile ion collides with an N$_2$O molecule. A standard basis of atom-centred one-electron functions of split-valence type, 3-21G, was employed which has the flexibility to describe changes in valence shells on ionization while offering acceptable computational economy. Second order Møller-Plesset perturbation theory corrections to Hartree-Fock energies were calculated (with core orbitals frozen).

RESULTS AND DISCUSSION

The DCT spectra showed five peaks identifiable as due to DEC reactions. From the translational energies corresponding to their maxima, five double-ionization energies were determined. The lowest, which corresponds to the ground triplet state of N$_2$O$^{2+}$, is 35.7 ± 0.4 eV which agrees with the value of 36.0 eV measured previously [3]. The value calculated in the present investigation for the double-ionization energy to the ground triplet state is 35.84 eV. The closeness of this to both the present and previously measured values confirms that the computational method used does for N$_2$O, as it did for CO$_2$, COS and CS$_2$ [7], accurately predict double-ionization energies to ground triplet states of the dication. The next highest double-ionization energy measured in the present investigation is 38.6 ± 0.4 eV. This is significantly higher than the previously measured ground singlet-state energy of 37.0 ± 0.2 eV [3] which has been calculated in the present investigation to be 37.09 eV.

The present data for the higher state energies, together with those calculated previously, are shown in Table 1. Where states with closely-lying computed energies are present, an average has been taken which is shown in the appropriate column. The experimental data have been positioned to correspond to those states having the most similar energies. It can be seen that the experimental data are not too different from those calculated by Price et al [3] and are in excellent agreement with those calculated by Connor et al [1]. This gives some confidence that triplet states were populated in the present DCT spectroscopy experiments and that, provided groups of calculated values similar to those shown in Table 1 are considered, calculated and experimental data are in excellent agreement.

Table 1: Energies (in eV) of triplet electronic states of N$_2$O$^{2+}$ measured in the present investigation and calculated previously [1,3]

<table>
<thead>
<tr>
<th>State(s)</th>
<th>$^3\Sigma^-$</th>
<th>$^3\Pi$</th>
<th>$^3\Delta$, $^5\Sigma^+$</th>
<th>$^3\Pi$, $^3\Pi$</th>
<th>$^5\Sigma^+$, $^5\Pi$</th>
<th>$^5\Pi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calc. [3]</td>
<td>35.7</td>
<td>38.1</td>
<td>41.0</td>
<td>43.1</td>
<td>45.8</td>
<td></td>
</tr>
<tr>
<td>Calc. [1]</td>
<td>35.7</td>
<td>39.2</td>
<td>41.7</td>
<td>42.8</td>
<td>46.5</td>
<td>49.2</td>
</tr>
<tr>
<td>Expt.</td>
<td>35.7</td>
<td>38.8</td>
<td>41.8</td>
<td>43.1</td>
<td>$\sim$47</td>
<td></td>
</tr>
<tr>
<td>(±0.4) (±0.4) (±0.4) (±0.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Computed data are adjusted to make 35.7 eV the $^3\Sigma^-$ state energy
b Average of calculated state energies

REFERENCES
**Investigation of Ligation Effects by Charge Stripping of FAB Produced Metal Ions**

Sæsl M. McCullough, A. Daniel Jones, and Carlito B. Lebrilla  
Department of Chemistry and Facility for Advanced Instrumentation, University of California, Davis, CA 95616

Formation of simple gas-phase metal-ligand complexes offers the opportunity to gain insight into the effects of ligation on the electronic energy levels of metal ions. The charge stripping (CS) of these complexes allows the determination of second ionization energies through the kinetic energy loss or $Q_{\text{min}}$, in the charge stripping process. These values, when compared with the second ionization energies of the corresponding bare metal ions, provide a measure of the degree of stabilization of a metal by a particular ligand.\(^1\)

We have used the combination of FAB/CS in a VG-ZAB 2F mass spectrometer to produce doubly charged metal and metal-ligand complexes of alkaline earth, transition and rare earth metals from their metal salts. Ions are selected with the magnetic sector, collided with $N_2$, and analyzed with the electrostatic analyzer. Values are obtained using the additive calibration method described by Beynon.

Table 1 shows a representative sample of the transition and lanthanide metals produced with this method. The $Q_{\text{min}}$ values obtained correspond well (within the experimental error of ±0.5 eV) to the literature values for second ionization energies. The charge stripping spectra of the bare metal ions (e.g. Co$^{2+}$, Figure 1a) contain a single peak with no high energy (lower $Q_{\text{min}}$) components representative of electronically excited ions.

The charge stripping of ligated metal ions (e.g. CoCl$^{2+}$, Figure 1b) produces similarly uncomplicated spectra. Ligation by either OH, O, or Cl increases the $Q_{\text{min}}$ values thereby making it more difficult to remove a second electron (Table 2). For example, the energy necessary to charge strip CoCl$^{+}$ is 0.9 eV greater than the corresponding value for Co$^{+}$. This is expected for electronegative ligands. There are clear variations when either the ligand or the metal is changed. A Cl ligand on Mg increases the $Q_{\text{min}}$ value from the bare to the ligated by 1.4 eV but the OH ligand produces only a corresponding value of 0.5 eV. Similarly an O ligand on V increases the $Q_{\text{min}}$ by 2.8 eV but produces a $\Delta Q_{\text{min}}$ of 5.1 eV for Ho. In general, we find that ligation affects the lanthanide metals the most. These results clearly illustrate that charge stripping can be used to determine the effects of ligation on the ionization potential of ligated metal ions.

The observed $Q_{\text{min}}$ values for the ligated metal ions may be used to estimate the bond dissociation energy of the ligated metal dication. Through the energy diagram shown (Figure 3) it is possible to obtain the dissociation energy if the corresponding energy for the monocation is known. Several bond energies of ligated metal ions have recently been obtained allowing us to determine the bond dissociation energy of CoOH$^{2+}$.\(^2\) As shown in the diagram, this complex dissociates to form a combination of Co$^{2+}$ + OH or Co$^+$ + OH$^+$. The pathways to form the metal dication is endothermic by 2.1±0.5 eV and the alternative pathway is exothermic by 2.2±0.5 eV.

Reference:
2. Buckner, S. W.; Freiser, B. S. *Polyhedron*, 1988, 7, 1583
Table 1.

<table>
<thead>
<tr>
<th>Metal Ion</th>
<th>$Q_{\text{min}}$ (this work) eV</th>
<th>2nd Ionization Energies a eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>V+</td>
<td>14.5</td>
<td>14.7</td>
</tr>
<tr>
<td>Mn+</td>
<td>15.5</td>
<td>15.6</td>
</tr>
<tr>
<td>Fe+</td>
<td>16.3</td>
<td>16.2</td>
</tr>
<tr>
<td>Co+</td>
<td>17.5</td>
<td>17.1</td>
</tr>
<tr>
<td>Ni+</td>
<td>18.2</td>
<td>18.2</td>
</tr>
<tr>
<td>Pd+</td>
<td>19.2</td>
<td>19.4</td>
</tr>
<tr>
<td>Eu+</td>
<td>11.3</td>
<td>11.2</td>
</tr>
<tr>
<td>Ho+</td>
<td>12.2</td>
<td>11.8</td>
</tr>
</tbody>
</table>


Table 2.

<table>
<thead>
<tr>
<th>Ligated Metal Ions</th>
<th>$Q_{\text{min}}$ (eV)a</th>
<th>$\Delta Q_{\text{min}}$ (eV)a,b</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgOH+</td>
<td>15.6</td>
<td>0.5</td>
</tr>
<tr>
<td>MgCl+</td>
<td>16.5</td>
<td>1.4c</td>
</tr>
<tr>
<td>VO+</td>
<td>17.4</td>
<td>2.8</td>
</tr>
<tr>
<td>VCl+</td>
<td>16.6</td>
<td>2.0f</td>
</tr>
<tr>
<td>CoOH+</td>
<td>18.5</td>
<td>1.0</td>
</tr>
<tr>
<td>CoCl+</td>
<td>18.4</td>
<td>0.9</td>
</tr>
<tr>
<td>HoOH+</td>
<td>13.2</td>
<td>1.0</td>
</tr>
<tr>
<td>HoCl+</td>
<td>15.4</td>
<td>3.2</td>
</tr>
<tr>
<td>HoO+</td>
<td>17.3</td>
<td>5.1</td>
</tr>
</tbody>
</table>

a) Values have deviation of ± 0.5 eV
b) $\Delta Q_{\text{min}} = Q_{\text{min}}$ (ligated) - $Q_{\text{min}}$ (bare)
c) Results are still preliminary and must be used with caution.
PHOTODISSOCIATION PHOTOIONIZATION MASS SPECTROMETRY OF ALKENES
S.E. Van Bramer and M.V. Johnston

INTRODUCTION:
In Photodissociation Photoionization Mass Spectrometry (PDPI/MS) the analyte is first photodissociated to form neutral fragments. These neutral fragments are then photoionized with coherent vacuum ultraviolet radiation. Because fragmentation is the result of neutral photodissociation rather than ionic fragmentation, the resulting mass spectrum is much different than that observed with EI.

An important difference between neutral and ionic fragmentation is that rearrangement barriers are frequently much higher for neutrals. This means neutral isomers are less likely to rearrange to a common structure. As a result isomers with identical ionic fragmentation are often distinguishable with PDPI/MS.

EXPERIMENTAL:
An excimer laser (193 nm) is used for photodissociation. The resulting neutral fragments are then softly ionized with 10.5 eV coherent VUV radiation produced by frequency tripling the third harmonic of a Nd:YAG laser in a rare gas mixture. Alternatively the output of a Nd:YAG pumped dye laser is mixed with residual 1064 nm radiation to produce tunable UV radiation. This is then frequency tripled in rare gas mixtures to produce coherent VUV radiation from 121 to 129 nm (10.2 to 9.6 eV) which is used to softly ionize the neutral photodissociation products.

WAVELENGTH EFFECT:
One problem with PDPI/MS is that the ionization step can also induce fragmentation of the molecular ion, complicating the spectra. By careful selection of the photoionization wavelength it is possible to reduce this fragmentation, greatly simplifying the PDPI spectrum.

The photoionization step can also induce fragmentation of the neutral photodissociation products. The use of longer photoionization wavelengths also reduces this secondary fragmentation. Comparison of 118 and 128 nm PI and PDPI spectra shows how the use of longer wavelength PI simplifies the PDPI spectra.

1-Heptene Photoionization

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>m/z</th>
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</thead>
<tbody>
<tr>
<td>118.2 (10.5 eV)</td>
<td>20 45 70 95 120</td>
</tr>
<tr>
<td>128.0 (9.7 eV)</td>
<td>20 45 70 95 120</td>
</tr>
</tbody>
</table>

1-Heptene PDPI/MS

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>193 nm PD</td>
<td>20 45 70 95 120</td>
</tr>
<tr>
<td>118 nm PI</td>
<td>20 45 70 95 120</td>
</tr>
<tr>
<td>193 nm PD</td>
<td>20 45 70 95 120</td>
</tr>
<tr>
<td>128 nm PI</td>
<td>20 45 70 95 120</td>
</tr>
<tr>
<td>29</td>
<td>20 45 70 95 120</td>
</tr>
<tr>
<td>41</td>
<td>20 45 70 95 120</td>
</tr>
</tbody>
</table>
FRAGMENTATION PATTERNS:

The PDPI/MS fragmentation of alkene isomers from C₂ to C₁₄, including dienes and branched alkenes, were studied with both 118 and 128 nm PI. Alkenes were chosen because they undergo rapid rearrangement after ionization, as a result their mass spectra are often identical. Unlike their conventional mass spectra the PDPI mass spectra of alkene isomers are very distinctive and the location of the double bond is readily determined. Previous photochemical studies indicated that 193 nm photodissociation would induce α-cleavage in alkenes. The resulting unsaturated allyl radical identifies the location of the double bond in the PDPI/MS. Apparently the saturated compliment undergoes further fragmentation to form the smaller radical species seen in PDPI/MS. The figure below shows the PDPI mass spectra (193 nm PD and 128 nm PI) for 1, 2, 3, and 4-Nonene. α-cleavage of 1-Nonene results in the fragment at m/z 41. α-Cleavage of 2-Nonene gives the peak at m/z 55. 3-Nonene has two α-cleavage sites, loss of C₄H₈ gives m/z 69 and a second cleavage, loss of CH₃, gives m/z 54. In 4-Nonene only cleavage at both α sites, m/z 54, is seen. The other alkene isomers studied undergo similar fragmentation.
Glycosphingolipids are important components of cell membranes. Their basic structure (1) comprises a carbohydrate group (R) attached to a ceramide residue. The ceramide group (R = H) is a long-chain sphingoid base, such as sphingosine (R' = CH=CH(CH2)3CH3) or sphingenine (R' = (CH2)4CH3), that is substituted at the amino group by a fatty acid (R'' = alkyl chain). If R were a monomer or polymer of a simple sugar, the molecule would be called a cerebroside. The structures of the carbohydrate group and the sphingoid and N-acyl chain can vary. Thus, easy but complete structural elucidation of ceramides and cerebrosides has traditionally been a challenge. Recently, however, fast atom bombardment (FAB) and collision-induced dissociation (CID) of (M + H)+ and (M - H)- ions has been shown to be an important avenue for their structure determination [1-3].

As an alternative to using CID of (M + H)+ and (M - H)- ions, we have been exploring the use of alkali metal ions as reagents to enhance structural determination by CID. Here, we show some results from applying CID of (M + Cat)+ ions, where Cat = alkali metal ion, to structure determination of ceramides and cerebrosides. All mass spectra were acquired by using a VG 70-S, forward geometry mass spectrometer. FAB matrices were 3-NBA and 3-NBA saturated with alkali iodides. CID occurred in the first field-free region, and CID spectra were acquired at 50% beam reduction with He. B/E linked scans were used to record the spectra.

Figure 1 shows CID spectra of (M + H)+, (M - H)-, and (M + Li)+ ions of a ceramide and a cerebroside.

![CID spectra of ceramides and cerebrosides](image)

Figure 1. CID spectra of (M + H)+, (M - H)-, and (M + Li)+ ions of N-oleyl sphingosine (A-C), and N-stearoyl dihydrolactocerebroside (D-F).
S and T ions in Figure 1C and 1F are analogous to those reported previously for \((M - H)^+\) ions [1]. CID of \((M + Li)^+\) ions gives complementary information to \((M + H)^+\) and \((M - H)^+\) ions, and more information about the structure of the \(N\)-acyl chain. Our detection limit for obtaining an interpretable CID spectrum of ceramides is 10 ng, which is comparable to that previously reported for \((M + H)^+\) ions [1].

Figure 2 shows CID spectra of \((M + Cat)^+\) ions, where Cat = alkali metal ion, of a ceramide. The spectra change in interesting ways with increasing size of alkali metal ion. The abundances of closed-shell ions, H, T, S, and V, decrease, while, abundances of open-shell ions, P, K, and \((T + 1)\), increase. For cerebrosides, somewhat similar chemistry occurs: Abundances of closed-shell ions, \(Y_0\), B, C, and S also decrease, while the abundance of the open-shell ion, \((J + 1)\), increases.

We do not entirely understand why the fragmentations change with increasing size of alkali metal ion. We hypothesize, however, that the changes may be a result of differences in multidentate metal ion binding to several binding sites.

REFERENCES
Reactions of ions and neutrals at keV collision energies have been extensively studied and attempts to understand the processes that occur have rely on several different types of measurements, e.g. energy loss accompanying ion-neutral collision, what fraction of the energy loss appears as internal energy to affect dissociation, the time-scale of the dissociation process, etc. An alternative method for probing the dynamics of collisional activation is to measure the neutrals formed by keV collisions. One of the questions that remain is the importance of endothermic charge exchange. A magnetic sector (EB)/reflectron time-of-flight instrument offers a unique opportunity for the study of the neutrals. The ability of the EB/reflectron time-of-flight to study neutrals is based on a detector situated behind the reflectron. Voltages may be placed on the reflectron such that only neutrals can reach the detector situated behind the reflectron.

Figure 1 shows the ion arrival time distribution of the mass-selected (M+H)' ion of sinapic acid on the detector position behind the reflectron (no voltage applied to the reflectron). Figure 1 also shows the arrival time distribution of the neutrals formed upon collisions with He (4 keV collision energy; 25% beam attenuation). It should be noted that the neutral abundance can not be accounted for by CID. The high abundance of neutrals can only be accounted for by neutralization of the ions by endothermic charge transfer reactions. This result indicates a significant portion of the collision energy is converted into internal energy of the collision gas, because the ionization potential for He is 24.6 eV.

A unique experiment to the magnetic sector(EB)/reflectron time-of-flight experiment is neutral-ion correlation. The experiment involves the use of a neutral detector to start the time of flight clock for ion detection. Since the start is caused by a neutral formed by an ion process, for example metastable decay, an ion that dissociates into daughter ions and daughter neutrals produces a signal for the daughter ions. Ions that lose their charge to the target gas, for example endothermic charge transfer, will produce a neutral signal without producing an ion signals. The correlation should therefore distinguish charge transfer neutrals from neutrals that produce fragment ions. Ideally, metastable spectra should give an ion for each neutral; however, the metastable spectra obtained by neutral-ion correlation experiments give only a small percentage of the numbers of ions for a particular starting neutral. For example, a metastable spectra was obtained with 100,000 neutral starts; however, only approximately 500 ion counts were observed. This poor collection efficiency of the daughter ions may be attributed to three factors.

1. Ions decaying in the reflectron will produce neutrals without a correlated ion (Flight time of ion and neutral will depend on the position of decomposition).
2. Ions can undergo collision with the background gas to produce a neutral without a correlated daughter ions.
3. Inefficient collection of ions by the short co-linear reflectron.

The effects of neutralization can be observed in the neutral-ion correlation experiment. The large number of uncorrelated neutrals produced by endothermic charge transfer limits the signal to noise ratio of the CID spectra by this method.
Arrival Time Distribution of Ions and Neutrals of Sinapic Acid (M+H)+ using the Line of Sight Detector.
He Collision Gas at 5 x 10^-4 Torr.

Figure 1.
Fundamental Studies and Optimization of Anthracic Acid
as a Matrix for Protein Analysis by $^{252}$Cf-PDMS

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We have recently introduced the use of small molecules as matrices in $^{252}$Cf plasma desorption mass spectrometry (PDMS)\textsuperscript{1} as an alternative to the commonly used polymeric matrices, such as nitrocellulose. One of the most interesting of the new matrices is anthracic acid (AA), which gives much sharper protein molecular ion peaks than are obtained with nitrocellulose. In addition, the background in the molecular ion region is reduced by over 50\% when anthracic acid is used as the matrix instead of nitrocellulose. We have pursued studies of the anthracic acid matrix to optimize the conditions for high molecular weight protein analysis. Further, fundamental studies have been done to increase knowledge concerning the desorption mechanisms in $^{252}$Cf-PDMS.

The optimization studies included determining the effect of protein solution and matrix parameters on the molecular ion yield of bovine insulin (SBI). In these studies, a cover glass was used to spread the SBI solutions over the electrosprayed substrates in order to ensure uniform coverage of small volumes over the surfaces. For each study, adsorption times were 5 minutes, following by rinsing of the targets with purified water and spinning dry at 10,000 RPM. In the first study, thicknesses of the AA substrate were varied by electrospraying different volumes of the solution (2 g/L in acetone). The thicknesses were determined by measuring the energy loss of alpha particles passing through the deposits. A plot of the results is shown in Fig. 1. Optimum SBI ion yield is obtained at an anthracic acid thickness of about 100 ng/cm$^2$. For the remaining studies, electrosprayed AA deposit thicknesses of 100 (±10) ng/cm$^2$ were used. The results of the SBI concentration studies are shown in Fig. 2. The volume of SBI solution applied for each target was 5 µL. The SBI yield increased strongly with the concentration of solution applied. The ratio of singly to doubly charged molecular ions increased with solution concentration as well. Studies in which the volume of SBI solution (10$^{-3}$ M) was varied from 1 to 5 µL showed no significant change in SBI intensities.

As part of the fundamental studies, a radio-assay using $^{14}$C-lysozyme revealed that about six times more protein is adsorbed onto a nitrocellulose (NC) matrix than an AA matrix prepared under the same conditions, though molecular ion yields from two such targets are similar. In another fundamental study, the fragmentation pattern of bradykinin (BRN) was studied for BRN adsorbed onto NC and AA. A plot of the intensities of some of the observed fragment ions relative to the BRN molecular ion is shown in Fig. 3. In general, fragment ion intensities are significantly decreased for the AA matrix, compared to the NC matrix. In addition, side chain fragmentation, such as the "d" series, are less sensitive to the substrate used, while the "a" series fragments are very much affected. Fig. 4 shows the axial kinetic energy (KE) distributions for BRN adsorbed onto AA and NC. These measurements were made using the method developed by Jacobs\textsuperscript{2}. The average axial KE is lower when AA is used (2.2 eV) than when NC is used (3.8 eV). The curves represent a maxwell-boltzmann fit to the data. Finally, angular correlation measurements were made for BRN/AA using the method developed by Mudgett\textsuperscript{3}. The angle of $^{252}$Cf fission fragment incidence was varied by varying the source to target distance and using an annular collimator over the target. Data was compared to computer simulations to determine the angle of emission and total (axial + radial) initial KE of ions. The total initial KE was found to be 4 keV for BRN/AA, and 10 keV for BRN/NC. For both targets, the angle of emission of BRN was determined to be 60° with respect to the incident fission fragments.
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Fig. 1.

SBI/AA THICKNESS STUDY

![Graph showing SBI intensity vs. substrate thickness (μg/cm²)]

Fig. 2.

SBI CONCENTRATION/AA

![Bar chart showing SBI concentration (M) vs. relative intensity, with data points for [(M+H)+] and [(M+2H)+].]

Fig. 3.

Fragment Ion Intensities Relative to the (M+H)+ of Bradykinin

![Graph showing relative intensity of fragment ions, with data points for AA and NC labeled.]  

Fig. 4.

BRADYKININ/ANTHROIC ACID

![Graph showing the distribution of kinetic energy (eV) for Bradykinin/Athyroic Acid, with the average KE = 2.2 eV and <E>=2KT, T = 12,900 degrees.]  

BRADYKININ/NITROCELLULOSE

![Graph showing the distribution of kinetic energy (eV) for Bradykinin/Nitrocellulose, with the average KE = 3.8 eV and <E>=2KT, T = 22,100 degrees.]  

Are Large Highly Charged Polyatomic Ions Stable?

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According to electrostatic theory, like charges repel each other. Therefore, placing many charges on a polyatomic ion tends to destabilize it. We have modeled the effect of coulombic energy on the dissociation of large highly charged polyatomic ions using a simple electrostatic contribution superimposed on a "typical" potential curve for dissociation of a chemical bond. The calculation was oriented toward biopolymer ions modeled as thin strings containing a set of evenly spaced elementary charges. The "chemical" part of the bonding model consisted of a Morse curve parameterized to give a 3.25 eV bond dissociation energy and a 400 mdyne/Å force constant. Based on the results from this model, many of the highly charged ions observed in electrospray ionization mass spectrometry (ESI-MS) are probably energetically unstable against dissociation into two or more charged fragment ions. For example, in our model an ion of 550 Å length containing 26 elementary charges should be unstable by almost 9 eV, yet the +26 charge state of myoglobin (estimated "extended" length ~550 Å) is readily observed in electrospray mass spectra. The fact that highly charged but undisassociated ions are often observed in ESI-MS might be attributable to the long-range nature of the coulomb interaction versus the short-range nature of "chemical bonding" interactions. Thus, coulombic repulsion is relatively inefficient in reducing the barrier to dissociation, even though the overall energy balance may favor dissociation, making the system "metastable". For example, in the system described above the energy barrier was lowered by only 0.32 eV even though the overall energy balance favors dissociation by almost 9 eV. The slight lowering of the dissociation barrier that does occur may, under certain circumstances, significantly alter (accelerate) the kinetics of dissociation. This may help explain certain recent findings concerning the thermally induced dissociation of highly charged biomolecular ions in which the more highly charged ions exhibited reduced stability.

The modeling studies show several trends: Coulombic energy lowers the dissociation energy with an approximately quadratic dependence on total charge, with an order of magnitude of ~10 eV for physically realistic systems. The barrier to dissociation is lowered quadratically with total charge. Barrier lowering may amount to as much as a few tenths eV in physically realistic cases. There is an increasing tendency for highly charged ions to fragment near the center of the polymer chain. (Simple RRKM arguments favor terminal fragmentation for uncharged systems.) Finally, if polymer chain length and charge are both increased so as to keep m/z constant, the amount of barrier lowering grows approximately logarithmically with the size of the system.

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Figure 1. Potential energy curves for the dissociation of a string-like ion containing various numbers of charges. The curve labeled (13, 13, 550) for example, corresponds to an ion of 550 Å chain length and 26 total charges breaking into equal sized fragments with 13 charges each.

Figure 2. Energy barrier lowering as a function of charge state for a 550 Å long ion.

Figure 3. Dissociation energy as a function of charge state for a 550 Å long ion.
We have been working on a procedure for the characterization of unsaturated fatty ester moieties in the liposidomycin series of antibiotics, using an approach dependent entirely on mass spectrometry (1). After attempting several methods, we decided to opt for a recently established procedure, the charge-remote fragmentation of lithiated fatty acid cations as described by Adams, Gross, et al. (Refs 2-4). The adequacy of this procedure was easily confirmed on our instrument using low energy CID of a series of underivatized model fatty acids.

High resolution mass measurements proved that liposidomycin A contained two additional carbon atoms in the fatty side-chain as compared to liposidomycin B; NMR work done at RIKEN further suggested that the unsaturation occurs in the form of a CH₂-interrupted diene. Previous mass spectral work on the liposidomycins employed a strong acid FAB dopant in order to facilitate its degradation. In the present case, however, it was of utmost importance that this delicate functionality remain unrearranged throughout the experiments. Therefore, we endeavored to obtain structural information on the fatty acid moiety using the intact antibiotic in the positive-ion FAB, low energy CID of lithiated fatty acid fragments formed in the primary beam.

In Gross and Adams's original work, optimal conditions for many fatty acid charge-remote fragmentations (CRF) were obtained using meta-nitrobenzyl alcohol (3NBA): LiI. We found, however, that liposidomycin A performs poorly with this matrix, but that it does give good spectra from LiBr:glycerol.

We found that the abundances of free fatty acid fragments derived from intact compound were too low to permit detection of CRF, but that the abundance of the dehydrated fatty acid was sufficient to permit MS/MS of its lithiated cations. We were thus able to detect sufficient CRF to deduce the presence of 7,10-hexadecadienoic acid -- an unusual fatty acid -- in the liposidomycin A molecule. The double bond configurations remain as yet undetermined.
Instrumental conditions: VG 70 SEQ, primary beam generated by FAB (Xe), 8 kV; collision gas Kr (50% beam reduction) at collision energies ($E_{\text{lab}}$) of 10-75 eV.

LASER DESORPTION VERSUS FAST ATOM BOMBARDMENT: THE EFFECT OF ION GENERATION PROCESS ON SURFACTANT ANALYSIS RESULTS


The utility of Fast Atom Bombardment (FAB) mass spectrometry for the analysis of many ionic surfactants has been well documented over the years. Many commercially available surfactants are mixtures of several different components or oligomers of a specific surfactant. Hence determination of both the chemical composition and molecular weight distribution of the surfactant components are necessary. Component identification in surfactant mixtures can often be obtained by using FAB, however, the use of a liquid matrix often complicates the analysis and some surfactants are not amenable to analysis by FAB.

Laser Desorption (LD) mass spectrometry combined with Fourier transform mass spectrometry offers many advantages for surfactant analysis including the elimination of the liquid matrix, high sensitivity, laser desorption/postionization to obtain library-searchable EI mass spectra of surfactants, the high resolution measurement of the parent ions and the accurate mass measurement of parent and fragment ions.

This study was designed to: investigate the usefulness of LD/FTMS for cationic and anionic surfactant analysis, to compare FAB versus LD mass spectra for mixture analysis (component abundance profiles) and to show the effect of LD acquisition parameters (such as laser power and postionization) on surfactant analysis results.

The LD experiments were performed by using a 3T EXTREL FTMS-2000 mass spectrometer equipped with a high-power pulsed CO$_2$ (10.6 um) laser, to ablate and ionize the surfactants. The output energy of the laser was controlled by adjusting an aperture and was estimated to be on the order of 0.05 J per pulse for the laser desorption/ionization results. For LD/EI postionization experiments, the laser energy was adjusted below the laser ionization threshold to desorb/ablate surfactant molecules that were subsequently ionization by using electron ionization. The FAB experiments were performed by using a Kratos MS-50 HMTA mass spectrometer. Both commercial surfactant mixtures and reference standards were analyzed by both FAB and LD.

Positive- and negative-ion spectra of typical surfactants obtained under FAB and LD ionization are similar and illustrated by mass spectra of a dialkyldimethylquaternary amine salt (Figures 1a & 1b)

For certain surfactants the LD technique appears to yield higher molecular ion abundances by avoiding the use of a sample matrix. The FAB spectrum (Figure 2a) contains abundant low mass matrix ion (m/z 131, 185, etc.) while the LD spectrum (Figure 2b) is essentially only the molecular cation.

In these laser desorption/ionization experiments, molecular ion abundance is strongly dependent on the output power of the laser. In a preformed ion like the dialkyldimethylquaternary amine, higher laser power results in increased fragmentation (Figures 3a & 3b).
The results of these experiments suggest that FAB and LD ionization techniques yield similar positive- and negative-ion mass spectra of typical ionic surfactants. Additional experiments need to be conducted on laser desorbed ions including high resolution daughter ion measurements for composition determination, breakdown curves and energy variation.
INTRODUCTION

Cooks and coworkers have illustrated several processes that occur when mass-selected polyatomic ions collide with a surface. These include (i) elastic scattering, (ii) inelastic scattering (surface-induced dissociation; SID), (iii) charge exchange and (iv) chemical reaction. Chemical reaction at the surface, which occurs primarily for odd-electron ions, is illustrated by the ion of m/z 91 in the reported spectrum for benzene (m/z 78)\(^1\). The ion of m/z 91 has been proposed to correspond to addition of methyl followed by loss of H\(_2\).\(^1\) We report here the spectra that result when benzene, benzene-d\(_6\), pyridine, and pyridine-d\(_6\) collide with a surface in our 90° tandem quadrupole mass spectrometer. Comparison is made between gas-phase and surface reactions. The goal of the research is to determine the mechanism(s) of alkyl ion addition at the surface.

EXPERIMENTAL

The instrument used in this investigation consists of two Extrel 4000 amu quadrupoles arranged in a 90° geometry. For this investigation, ions were produced by electron ionization and chemical ionization. An ion of interest is mass-selected with the first quadrupole and allowed to collide with the surface. The potential differential between the ion formation region and the surface determines the kinetic energy of the ion/surface collision. Surfaces used in the investigation include bare metals with adventitious hydrocarbon adsorbates (from pump oil) and highly-ordered, self-assembled monolayer films of alkanethiols on gold. Product ions are analyzed by the second quadrupole and a conversion dynode electron multiplier is used for ion detection. A Teknivent Vector/Two data system is used to acquire and process the data.

RESULTS FOR BENZENE

The spectra that result when benzene and benzene-d\(_6\) are mass-selected and allowed to collide with a surface are shown below (Fig. 1 and 2). The spectral regions that correspond to alkyl ion addition are shown in greater detail in Fig. 3 and 4. Peaks corresponding to addition of 1-4 carbons upon ion/surface collision are evident in these spectra. The number of H (or H and D) in the reaction product ions varies with the collision energy (Fig. 3 and 4). Fig. 5 indicates that alkyl addition products dissociate by losses of combinations of H, D, and C to form lower mass product ions with varying numbers of H and D. These ions appear in the spectrum along with those ions that are direct dissociation products of C\(_6\)H\(_6\)\(^+\).

RESULTS FOR PYRIDINE

The spectra that result when pyridine and pyridine-d\(_6\) are mass-selected and allowed to collide with a surface indicate the presence of product ions corresponding to alkyl ion addition. The pyridine spectra show a greater relative abundance of the ion corresponding to addition of an intact methyl group than do the benzene spectra. This may result from the probability of pyridine to undergo methylation at nitrogen, whereas benzene must undergo methylation on the ring (with ring opening and H\(_2\) loss likely).

MECHANISM FOR ION/SURFACE REACTION

It has been proposed that benzene radical cation undergoes alkyl addition at the surface by the mechanism illustrated below.\(^2\) The mechanism is supported by the fact that benzene radical ions do not undergo alkyl ion addition when mass-selected and allowed to collide with alkanes in the second quadrupole of a triple quadrupole instrument.\(^3\) The low reactivity of benzene radical ions in gas-phase reactions has been noted by other workers.\(^3\)

The proposed first step in the ion/surface reaction of C\(_6\)H\(_6\)\(^+\) is neutralization by electron transfer from surface species to form neutral benzene and an ionized surface adsorbate. The resulting neutral benzene is then re-ionized by addition of a proton or an alkyl ion. This initially formed ion can then fragment by loss of H\(_2\) or by C\(_6\)H\(_6\) loss to form lower mass fragments. Our results for benzene-d\(_6\) are consistent with this mechanism. For example, the ion at m/z 91 for benzene shifts to m/z 98 and 97 for benzene-d\(_6\) (see Fig. 1 and 2). This can be envisioned as addition of CH\(_3\)\(^+\) followed by loss of HD or H\(_2\). Our results for gas-phase reactions of benzene support this mechanism and suggest that the mechanism shown earlier could be written more generally to include addition of R\(^+\) followed by loss of RH, where R represents an alkyl ion or H.

SURFACE-INDUCED DISSOCIATION OF SINGLY- AND MULTIPLY-PROTONATED PEPTIDE IONS
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Amino acid sequence determination by tandem mass spectrometry is a rapidly expanding field. The majority of these investigations have been performed by low and high energy gas-phase collision-induced dissociation (CID). A new technique, surface-induced dissociation (SID), has been shown to deposit high average internal energies in a controllable manner (1). One goal of this investigation is to determine if the types and relative abundances of sequence ions from singly- and doubly-charged parent ions can be controlled by varying the collision energy in the surface-induced dissociation experiment. Another goal is to determine if side-chain specific ions of type d and w are detected in the SID spectra (2). These ions are generally detected in gas-phase collisional activation experiments performed in the high-energy regime (keV), but not in experiments performed at low laboratory collision energies.

The instrument used in this investigation consists of two Extrel 4000 amu quadrupoles arranged in a 90° geometry. For this investigation, ions were produced by Cs⁺ bombardment (LSIMS) or electrospray (see below).

One objective of this investigation is to determine the effect of collision energy on the types and relative abundances of sequence ions. Figure 1, the 10 eV SID spectrum of the singly charged peptide GYLTLYKYKASA, is an example the effect of collision energy on the relative abundance of sequence ions. The low mass fragment ions increase in abundance as the energy of the collision increases. The high mass fragment ions and the parent ion are barely present at 30 eV and 65 eV collision energy (data not shown). Reducing the SID collision energy to 10 eV produces a full series of b type ions in the spectrum.

The SID experiments were also performed on doubly-charged peptide ions. 30 eV SID of the doubly-charged peptide, angiotensin I, produced a series of a and b type sequence ions in the spectrum (data not shown). Also present in the spectrum of the doubly-charged parent, is a singly-charged ion corresponding to the intact peptide. Further experiments are required to determine the nominal mass of this ion. SID spectra of the doubly-charged peptide Substance P illustrates the effect of collision energy on the abundance and type of fragment ions (data not shown). At low collision energies, 15 eV, ions of type b are present; as the collision energy increases ions of type a become more abundant.

Another goal of this study is to determine if side-chain specific ions of type d and w are detected in SID spectra of peptides. Figure 2 shows the 500-1050 m/z range of the 60 eV SID spectrum of the peptide Substance P. Side-chains specific ions, labelled d₃ and d₆, correspond to the loss of a glutamine side chain.

The electrospray ionization source used in this investigation was adapted from Chait and coworkers and Knapp and coworkers (3,4). A 40 eV surface-induced dissociation spectrum of the doubly-charged ion derived from des Arg¹ Bradykinin is shown in Figure 3. A series of y type ions is present in the spectrum.

SUMMARY
* High average internal energy deposition is apparent from the extent of fragmentation of the peptide ions investigated.
* The types and relative abundances of sequence ions from both singly- and doubly-charged parent ions are dependent on the ion structure and the SID collision energy.
* Ions of type d and w are detected in the SID spectra.

As part of the development of an organic ion microprobe, to be used for imaging of particular organic compounds in biological tissue, various methods of quadrupole-based tandem mass spectrometry (MS/MS) have been investigated. High transmission efficiency is essential for the success of the organic ion microprobe, due to expected low analyte concentrations in biological tissue and the potential for sample damage from prolonged exposure to the primary ion beam. MS/MS is necessary for organic ion imaging because of the complex nature of the biological matrices. The goal of these studies was to optimize the efficiency of daughter ion production and transmission by first determining daughter ion properties and then designing ion optics based on those properties. The properties of main interest are daughter ion kinetic energy and angular distribution.

Surface induced dissociation (SID) as a means of MS/MS has several potentially attractive features over gas-phase collisional activation dissociation (CAD) commonly used with the triple quadrupoles. One advantage is the reduction in gas load and therefore pumping requirements. A more subtle advantage is mitigation of the ion optical depth-of-field problem inherent in gas phase collisions—daughter ions originate from a planar surface of well defined potential. To design and construct an efficient lens system to collect and transport daughter ions into the second stage of mass analysis, however, it is essential to know the kinetic energy and angular distributions of the daughter ions.

A retarding grid analyzer was used for these measurements, because retarding analyzers do not exhibit ion optical discrimination, as does an electrostatic sector. A lens system was constructed to direct the parent ion beam exiting the first quadrupole into the collision surface in a controllable manner. The system consisted of an acceleration lens, which was used to vary the collision energy, and tandem sets of x,y deflection plates which were used to vary the collision angle, while keeping the ion beam very near the mean spectrometer ion axis at the plane of collision. Daughter ions should exhibit energy distributions peaked at lower energies than the parent ions, due to the reduction in mass upon fragmentation, as well as the conversion of kinetic energy to internal energy in the fragmentation process. Since there is no mass analysis with this type of analyzer, however, energy distributions of inelastically scattered parent ions can obscure the interpretation of the results.

Collisions of acetone molecular ions (m/z 58) ions with: (1) a microchannel plate; (2) a small stainless steel tube; and (3) a stainless steel mesh (50% transmission) were studied. Microchannel plates have been demonstrated as effective fragmentation surfaces at higher energies (1), and collisions with stainless steel surfaces have been used in quadrupole-based systems (2), and in a Fourier-transform mass spectrometer (3). We found, however, that only by severely attenuating the total transmitted ion current, were we able to observe energy distributions that differed from that of the parent ion beam very near the mean spectrometer ion axis at the plane of collision. Daughter ions should exhibit energy distributions peaked at lower energies than the parent ions, due to the reduction in mass upon fragmentation, as well as the conversion of kinetic energy to internal energy in the fragmentation process. Since there is no mass analysis with this type of analyzer, however, energy distributions of inelastically scattered parent ions can obscure the interpretation of the results.

These results led us to conclude that SID was not an acceptable means MS/MS for our microprobe.

Subsequently, we installed a triple quadrupole system; preliminary measurements indicate that transmission (25% in mass selected mode for both quadrupoles with unit mass resolution) and fragmentation efficiencies (20% of...
transmitted parent beam with modest gas pressure) are much higher than for our SID system. Our secondary ion source injects a tightly focused and centered beam into the first quadrupole [4], which we believe contributes to our high efficiency. We previously demonstrated [5] that if a tightly focused beam enters a quadrupole mass analyzer on axis, the exiting beam also has a small circular cross section. Consequently, the beam remains well defined throughout the triple quadrupole system, enhancing transmission. Beam blow up from scattering in the middle "collision cell" quadrupole is suppressed by keeping ions very close to the mean ion axis.


Figure 1. Retarding-grid spectra for (a) parent ion beam and (b) ions following glancing collisions in a microchannel plate (x 100). The integrated intensity shown in both plots represents the intensity of all ions having kinetic energy sufficient to be transmitted through the grid, held at the potential indicated by the abscissa. Differentiation of retarding grid spectrum, shown only in (a) gives the kinetic energy distribution normal to the grid plane.

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SURFACE-INDUCED DISSOCIATION AT SELF-ASSEMBLED MONOLAYER ORGANIC SURFACES

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C.E.D. Chidsey, AT&T Bell Labs, Murray Hill, NJ 07974

Surface induced dissociation (SID) has proven to be an effective means for depositing large amounts of internal energy into polyatomic ions. The internal energy distribution is usually quite narrow and the amount of energy deposited increases rapidly over collision energies ranging from 0 to a few hundred eV. A major competitive channel to SID is charge exchange (neutralization) with the target. This limits the efficiency of surface induced collisional activation.

We have recently studied the effect of thiol-bound alkyl monolayer target surfaces on SID efficiency and internal energy deposition using a BEEQ hybrid instrument. The three targets used were (i) fluorinated and (ii) deuterated thiol-bound alkyl monolayers on gold and (iii) untreated stainless steel.

SID efficiency refers to the total fragment ion current divided by the total parent ion current colliding with the target. Experiments performed thus far have shown that the SID efficiency on alkyl monolayer surfaces is significantly higher than on stainless steel. Figures 2 and 3 show the relative SID efficiency for benzene and limonene on each target. In experiments where benzene was used, the efficiency of the fluorinated surface was 3 times greater than that of stainless steel. In the case of limonene, the fluorinated surface provided a ten fold increase. The deuterated surface was also more efficient than stainless steel, although not to the same extent as fluorinated surface. We attribute these results to the fact that less neutralization occurs at the alkyl-monolayer covered surfaces; the fluorinated surface having the best SID efficiency due to its high ionization potential.

In addition to yielding different SID efficiencies the surfaces give different SID mass spectra. These differences are due to both ion surface reactive collisions and differing internal energy deposition. Figure 4 provides a comparison of the fluorinated and stainless steel surfaces. At the low collisions energies studied, the fluorinated surface produces more lower mass fragments and hence appears to deposit more internal energy than the stainless steel surface. The 15eV spectrum of limonene on the fluorinated surface in Figure 4a resembles the 25eV SID spectrum on stainless steel in Figure 5.

References


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Figure 1. Illustration of self assembled monolayer surfaces.

Figure 2. Relative SID efficiency of benzene as a function of the target surface.

Figure 3. Relative SID efficiency of limonene as a function of the target surface.

Figure 4. SID mass spectrum resulting from limonene colliding with the a) fluorinated and b) stainless steel surface at 15eV.

Figure 5. The SID spectrum of limonene on stainless steel at 25eV.
INTRODUCTION

The extensive work of R.G. Cooks and coworkers in the area of polyatomic ion surface collisions has led to our interest in the use of surface-induced dissociation (SID) as a tool to probe the structure of large ions (e.g. clusters and peptides). In SID, mass-selected parent ions are activated and fragmented by collisions with a surface; a significant fraction of the lab frame collision energy (10-20%) is converted to internal energy. Thus, SID may represent a relatively simple way to activate large ions in instruments that operate at relatively low voltages. A factor that will affect applications of SID is sensitivity. One parameter that affects sensitivity is the fragmentation efficiency (sum of the daughter ion abundances divided by the mass-selected transmitted parent ion abundance). Several processes fundamental to ion-surface collisions affect the efficiency, including: the extent of elastic scattering and inelastic scattering, and the amount of reactive scattering. The relative amounts of elastic and inelastic scattering may be influenced by energetic, chemical and instrumental factors. Reactive scattering includes charge reduction, ion-surface reactions, and reactive sputtering, and will be affected by the chemistry of the ion and the nature of the surface. We have undertaken a comparison of fragmentation yields in SID on different instrumental designs, as well as a comparison of some the chemical properties that may affect fragmentation yields in SID.

EXPERIMENTAL

Two of the designs were implemented on a commercial triple quadrupole instrument (Finnigan TSQ-70). The first of these designs is an in-line device (consisting of a flat plate acting as an off-axis surface and a slanted deflector to force ions into the surface) which replaced the collision quadrupole of the TSQ-70 and has been described previously. The second device was a modification of a design based on a deflection cone and cylinder, as published by Cooks and coworkers. This device was made small enough to fit between the first analyzer quadrupole and the collision quadrupole in the TSQ-70. The third instrumental design was a tandem quadrupole SID instrument at Virginia Commonwealth University, which has been described previously. Efficiencies were calculated based on the definition: Efficiency = ∑F/P0, where ∑F is the sum of the fragment ion abundances and P0 was the transmitted parent ion abundance. The transmission measurements were made by adjusting the surface and lens potentials such that the maximum amount of parent ion signal was generated.

RESULTS AND DISCUSSION

Table 1 shows a comparison of efficiency data obtained on the three different experimental designs for benzene, pyridine and the tetramethylammonium ion. The results indicate that the highest efficiencies were obtained on the 90° tandem quadrupole at VCU. Efficiencies were found to be considerably lower for the slant plate device at NRL, and further efforts were not made with this device. The energy deposition properties of the different designs were found to be very similar (as determined by comparisons of the collision energy dependent fragmentation of the thermometer molecule W(CO)6 on all three instruments). Thus, the differences in efficiency are thought to be related to ion optics. One possibility is that the collection optics are better in the 90° design, or there are systematic underestimations of the parent ion transmission for the 90° instrument.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Benzene (30 eV SID)</th>
<th>Pyridine (30 eV SID)</th>
<th>(CH3)4N+ (30 eV SID)</th>
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</thead>
<tbody>
<tr>
<td>Slant-plate (NRL)</td>
<td>&lt; 0.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cone-cylinder (NRL)</td>
<td>0.2%</td>
<td>0.6%</td>
<td>2.7%</td>
</tr>
<tr>
<td>90° tandem quad (VCU)</td>
<td>5.5%</td>
<td>2.4%</td>
<td>7%</td>
</tr>
</tbody>
</table>

Table 1. SID efficiencies for three compounds on three experimental designs.
The results also suggest that there are significant variations fragmentation efficiency with chemical structure. Table 2 summarizes SID efficiencies for various compounds as obtained on the in-line (cone-cylinder) device at NRL. Efficiencies range from 0.2% for benzene to nearly 10% for some tetraalkylammonium ions. The results also directly compare the efficiencies for some similar odd and even electron ions. In general, the SID efficiencies are higher for even-electron ions. This effect may arise because the even electron ions have lower electron affinities, and therefore are less easily neutralized. As shown by the Wysocki group and the Cooks group, modification of the surface work function with bound organic monolayers also affects efficiency, and may be related to neutralization effects. At present, the significant differences between the efficiencies at 30 eV and 50 eV are unexplained. However, they may be the result of focussing problems with the in-line device as the collision energy increases.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Efficiency (30 eV)</th>
<th>Efficiency (50 eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene M⁺</td>
<td>0.2%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Benzene [M+H]⁺</td>
<td>0.8%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Pyridine M⁺</td>
<td>0.6%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Pyridine [M+H]⁺</td>
<td>4.8%</td>
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</tr>
<tr>
<td>Acetone M⁺</td>
<td>0.4%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Acetone [M+H]⁺</td>
<td>0.8%</td>
<td>0.6%</td>
</tr>
<tr>
<td>(CH₃)₄N⁺</td>
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<tr>
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<td>6.9%</td>
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<td>10.5%</td>
<td>4.0%</td>
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Table 2. A comparison of SID efficiencies for various compounds on the in-line device at NRL.

An example of the effect of surface neutralization is observed in the SID spectrum of C₆₀ (Figure 1). The singly charged fragment does not dissociate at collision energies of up to 130 eV, while the doubly charged ion does fragment at 130 V (260 eV). However, the only fragments observed are singly charged ions, as a result of the charge reduction process. Similar effects most likely play an important role in all SID efficiencies.

Figure 1. 130 V SID spectrum of C₆₀⁺ and C₆₀²⁺.

Surface-Induced Dissociation by FTMS: An Internal Energy Deposition Study

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Introduction
Surface-induced dissociation (SID) first introduced by Cooks in 1985, is a promising new technique for ion activation in connection with structural analysis studies. A limitation to collision induced dissociation (CID), is that the yield of the daughter ions decreases as the parent mass increases. This is a result of increasing mismatch in parent and target masses and the greater number of vibrational modes available in a larger molecule, resulting in enhanced internal energy distribution, instead of dissociation. Therefore, using a technique such as SID, no additional gas load is used, and a higher percentage of translational energy is converted into internal energy, due to the relatively infinite mass target.

This paper reports the first step in the characterization of SID by FTMS by a comparison study of the internal energy deposition in iron pentacarbonyl for both SID and CID by FTMS.

Mass Spectrometer: Electron ionization (El) SID experiments of iron pentacarbonyl were performed with a FTMS-1000, upgraded with a standard Extrel FTMS-2000 dual cell (4.76cm cubic cell) and operated at a magnetic field strength of 2.9T.

Procedure: Ions were formed in the source cell transferred to the analyzer cell through the 2mm conductance limit. After transferring ions to the analyzer side, lower mass ions were ejected by multiple ejection sweeps. The cyclotron radius of the chosen parent ion was excited by an attenuated on-resonance cyclotron frequency. After this tickle event a +25V pulse was applied by the analyzer trap plate, repelling the positively trapped ions causing them to strike the conductance limit and fragment. The duration of this event was set to 1.5 times the calculated flight time for the parent ion, from the center of the cell to the conductance limit.

Results and Discussion
To characterize the internal energy depositing by SID by FTMS, the much studied compound, iron pentacarbonyl was chosen. The critical energies necessary for the loss of each successive carbonyl group were used to estimate the internal energy distribution. A relatively recently developed method for estimating the internal energy distribution was used. The relative abundance of a fragment is divided by the known critical energy interval. The energy deposition can be therefore estimated by the daughter ion spectrum. The relative abundance of these fragment ions at various SID energies resulted in the internal energy distribution, shown in Figures 1 and 2.

The SID of Fe(CO)5+ by FTMS is compared with SID with a triple quadrupole and with CID by FTMS. The quadrupole spectrum at 60 eV has an internal energy deposition of 6.6 eV or 11%, while the FTMS spectrum of 64 eV has an average internal energy deposition of 2.5 eV or 3.8%. The percent energy deposition difference, being due to the angle of incidence of the collision, since SID by FTMS is at a grazing angle, while the quadrupole has more of a near-normal angle of incidence. Both methods do show an advantage of SID to CID, which is a narrow energy deposition range. Collision induced dissociation of Fe(CO)5+ with Helium, has a broader energy deposition range, but more importantly, the percentage energy deposition with increased collisional energy decreases much more rapidly.
Conclusion

It has been shown that SID by FTMS converts a relatively high percentage of translational energy into internal energy. The limitation of the translational energy being a function of the cell dimensions and magnetic field strength, as in CID. The increased conversion efficiency of translational energy to internal energy makes SID more useful for fragmenting higher mass ions. Future work will be implementing SID to higher mass compounds and studying its applicability to complex structure analysis problems.

![Figure 1: SID spectra for different collisional energies.](image)

![Figure 2: Collector average internal energy vs. collisional energy.](image)

<table>
<thead>
<tr>
<th>Collisional Energy (eV)</th>
<th>Average Internal Energy (eV)</th>
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<tr>
<td>64</td>
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REFERENCES


ACKNOWLEDGEMENTS

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As a result of the known inefficiency of collision-induced dissociation for structural analysis of molecules with masses in excess of a few thousand, even when high energy CID is employed, it is of interest to develop alternative strategies for ion activation. This paper describes recent studies intended to evaluate the relative merits of two alternatives, surface-induced dissociation and photodissociation. Results of comparative studies of SID and PD for a laser-desorbed porphyrin and metalloporphyrin are discussed. Relative dissociation efficiencies are calculated from daughter ion spectra obtained using various SID energies and PD wavelengths and time durations. In addition, results from the combined use of PD and SID for MS/MS/MS studies are presented.

Experiments were performed using a 7 T Nicolet FTMS-2000 mass spectrometer. CO2 laser desorption experiments were performed using a Tachisto 215 pulsed CO2 laser. A Lambda Physik EMG 201-MSC excimer laser was used to pump a Lambda Physik FL-2001 dye laser for ultraviolet and visible photodissociation experiments. The dye laser was operated from 568 nm to 605 nm in the visible region (rhodamine 6G) and from 368 nm to 402 nm in the ultraviolet/visible region (QUI).

Samples of tetra(4-pyridyl)-porphyrin (TPyP) and tetraphenylporphyrin iron chloride (TPP FeCl) were dissolved in dichloromethane and portions of each solution were spotted onto a stainless steel probe tip and the solvent evaporated. Each spectrum resulted from the addition of four co-added transients from separate laser desorption events.

**TPyP studies**

Figure 1 contains the photodissociation spectra for TPyP [M]+ ions with m/z 618 at both a) 388 nm and b) 575 nm. Photodissociation at 388 nm results in more extensive fragmentation of the porphyrin, especially in the pyridyl ligands, as might be expected for higher energy photon activation (3.19 eV at 388 nm compared to 2.16 eV at 575 nm). Note that the dye laser was fired for 1 second to obtain the spectrum in a) and for 60 seconds to obtain the spectrum in b). The relative dissociation efficiencies were dependent upon both the wavelength employed and the length of irradiation time. Dissociation efficiency vs. irradiation time was plotted for photodissociation at ultraviolet (390 nm) and visible (581 nm) wavelengths. Relative efficiency values were obtained by dividing the sum of the daughter ion abundances by the initial parent ion abundance. The graphs indicate that efficiency generally increases with increasing irradiation time up to a point, and then levels off as time is increased further. SID for this same compound is shown in Figure 2 at four different collision energies. The abundances of daughter ions increases as a function of increasing SID energy. The dissociation efficiencies vs. collision energy were graphed for TPyP parent ions and the efficiency Increases up to a point and then decreases. LD/PD/PD and LD/PD/SID experiments were performed for TPyP. After isolation of molecular ions and photodissociation at 581 nm, the daughter ions with m/z 540 were subjected to either SID or a second stage of photodissociation at 581 nm. Photodissociation did not produce any granddaughter ions, while SID produced granddaughter ions resulting from loss of a second pyridyl ligand from the m/z 540 ions.

**TPP FeCl studies**

Photodissociation of TPP FeCl [M-Cl]+ ions using ultraviolet and visible radiation results in the production of abundant daughter ions resulting from loss of C6H5 and 2(C6H5) ligands. As observed with the previous sample, in order to obtain comparable photodissociation efficiencies at these two wavelengths, considerably longer irradiation times in the visible region are required than in the ultraviolet region. The ultraviolet and visible photodissociation efficiencies calculated for various irradiation times were graphed and the efficiencies appear to increase with increasing irradiation time and then level off, similar to the earlier TPyP results. Although the efficiencies tend to increase with time, these daughter ions...
ion spectra are not always better for structural analysis purposes. Frequently more non-specific fragment ions are produced at the expense of daughter ions more indicative of ion structure. SID dissociation efficiencies of [M-Cl]+ ions were calculated and plotted from spectra obtained at four different collision energies and are similar to those obtained for TPyP. In Figure 3a, parent [M-Cl]+ ions are photodissociated with 388 nm radiation and the daughter ions with m/z 512 subjected to either b) SID or c) 388 nm photodissociation. Photodissociation produces only one major type of fragment ion (m/z 485 corresponding to loss of C2H3) while SID results in the generation of more types of fragment ions corresponding to successive losses of hydrocarbons (probably resulting from fragmentation of the residual phenyl rings).

In conclusion, PD efficiencies were higher than dissociation efficiencies obtained by SID. PD could be performed at various wavelengths and the efficiency manipulated as a function of irradiation time. Longer times tended to produce more types of fragment ions and in greater abundances. However, in some cases, longer irradiation times also resulted in the production of new fragment ions, at the expense of other fragment ions, which were not necessarily as useful for structural analysis. SID has the advantage that it does not depend upon the absorption characteristics of the parent ion. Although efficiencies are much less, it is the technique of choice for cases where the absorbance of the ion limits the utility of PD.
A New "in-line" Surface Induced Dissociation (SID) for Hybrid Tandem Mass Spectrometers

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In tandem mass spectrometry a new and very promising technique for activating mass selected ions is based on the collision of the precursor ion beam with a solid surface. Up to now, most surface induced dissociation (SID) experiments reported in the literature have been performed with an angular arrangement of the MS analyzer [1]. Instruments of this type cannot be obtained commercially and an adaption of commercial mass spectrometers requires a fundamental modification of their geometry.

We wish to present here an "in-line" SID cell allowing the use of conventional hybrid MS/MS systems. The cell is placed between the deceleration lenses of MS1 and the RF-only quadrupole of MS2. It consists of a metal tubing which houses an insulated electrode (Fig. 1). A variable electrical potential (deflection voltage) can be applied to this electrode which causes a deflection of the ion beam traversing the cell tubing. The deflected ions collide with the metall surface of the latter. The fragment ions thus generated are collected by ion optical lenses, and the resulting daughter ion spectrum is recorded by MS2. When a OV potential is applied to this electrode the mass spectrometer can be used for conventional low energy gas collision experiments.

The varying amounts of energy transferred by collision result in an array of activated ions, which can be described by a distribution function. The relative fragment ion abundances of model substances can be used as an indicator for the band width, and the mean of the distribution curve provided the fragment ions are formed in a linear reaction sequence and their appearance energies are known [2].

Experiments with tetraethyl silane indicated a high mean energy of the activated precursor ions upon in-line SID. The spectrum obtained with a collision energy of 100 eV corresponding to mean energy of 10.5 eV is reported in Fig. 2. A comparison of the fragmentation pattern obtained in this way with those in the EI- and the low energy gas collision spectra demonstrated the narrow energy band width and the comparatively high mean energies of the ions activated by SID.

The internal energies and the collision energies show an approximately linear correlation. The efficiency of the energy transformation could be shown to be 10-11 of the kinetic laboratory energy between 20 and 200 eV.

The activation efficiency with respect to fragment 'formation' (fragmentation efficiency) has been defined as the ratio of the total of the fragment ion intensities after the ion beam deflection (IF), and the precursor ion intensities before activation (IP). SID efficiency = IF/IP. The fragmentation efficiency (example tetraethyl silane) depends strongly on the focusing of the ion beam (voltages of the ion optical lenses). For determination of the fragmentation efficiency the focusing was adjusted for maximal IF in SID experiments and for maximal IP without deflection. Values between 4 and 8 % were obtained. The potential of the technique is demonstrated for peptides up to mass 1300 u (Fig. 3). With a collision energy of 25 eV a pronounced fragment formation and a good resolution of fragment ions was achieved. Peptides yield structure specific fragment ions of the A, B, C+2 and Y+2 series. In addition, larger fragments of the inner part of the peptide chains and ions corresponding to the various amino acids can be observed.

Fig. 1: Longitudinal section of the "in-line" SID cell.

Fig. 2: 100 eV SID spectrum of Tetraethyl silane, mean internal energy of the activated precursor ion: 10.5 eV.

Fig. 3: 25 eV SID spectrum of Angiotensin.
THE FORMATION OF AMMONIUM IONS BY SURFACE IONIZATION
OF TETRAALKYL AMMONIUM SALTS

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INTRODUCTION

Surface ionization (SI) on oxide-free metal surfaces is a very selective ionization technique for the detection of quaternary salts from mixtures. At filament temperatures < 1000 K SI mass spectra show only the intact cation whereas higher filament temperatures induce fragmentation of the cation. NH₄⁺ ions were abundantly formed by SI of tetrabutyl, tetrapropyl and tetraethyl ammonium salts at elevated filament temperatures [1]. These ions are not present in the CID spectra of the cations of these salts. This prompted us to elucidate the origin of the ammonium ions.

EXPERIMENTAL

The ion source employed is shown in Fig. 1. The salt was evaporated from the sample holder and ionized on a platinum wire heated to temperatures between 900 and 1750 K.

RESULTS

Fig. 2 shows the SI spectrum of tetrabutyl ammonium iodide at a filament temperature of 1600 K. For this temperature the SI spectrum is similar to that obtained by CID of Bu₄N⁺ [2] with the exception of the abundant NH₄⁺ signal (m/z=18) in the SI spectrum. CID MS/MS/MS studies of the fragmentation behaviour of the primary decomposition products of Bu₄N⁺ reveal the fragmentation step 188 → 130 and are in support of a further step 130 → 74, again by loss of butene, while the tendency of Bu₃NH⁺ (m/z=186) to form iminium ions is low. No ions of the ammonium type were observed in the fragmentation of Bu₃N=CH-Prop⁺ (m/z=184) [2].

In order to elucidate the fragmentation pathway leading to the NH₄⁺ ion SI spectra of Bu₄NI were recorded at filament temperatures between 1100 and 1700 K. Fig. 3 shows the temperature dependence of the relative abundances of the fragment ions Bu₃NH⁺(m/z=186), Bu₂NH₂⁺(130), BuNH₃⁺(74) and NH₄⁺(18) which differ by the loss of one to three butene molecules from the intact cation.

DISCUSSION AND CONCLUSIONS

The intensity profiles of Fig. 3 show the typical shape expected for subsequent monomolecular decomposition reactions thus providing evidence for the formation of NH₄⁺ in SI by successive loss of alkenes from the intact cation. The occurrence of this multistep process in SI can be explained by the trapping of ions in a two-dimensional potential well on the filament surface arising from the image force on the ions, and the permanent heating of the ions during their lifetime \( \tau \) in the trap. For Bu₄N⁺ the depth of the well is about 0.7 eV at a distance of about 0.5 nm. Under the assumption of thermal equilibrium \( \tau \) can be estimated to about \( 10^{-8} \) to \( 10^{-9} \) s in the temperature range used in SI.

REFERENCES

The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Fig. 1: The thermionic source (schematic)

Fig. 2: SI spectrum of Bu₄NI (T_r1=1600 K)

Fig. 3: Relative abundance of the fragment ions m/z=186, 130, 74 and 18
A NOVEL COLLISION CELL FOR HIGH RESOLUTION ENERGY LOSS ION MOLECULE COLLISION PROCESSES

A Gareth Brenton and Christopher S Enos
Mass Spectrometry Research Unit, University of Swansea, Swansea SA2 8PP, U.K.

The study of ion molecule collisions (at kilovolt energies) has proved a difficult technique in providing clear spectroscopic information on ions and neutral species, primarily because of the very high energy resolutions that would be required [1] and also that the collision process tends to smear spectra, for several reasons. The technique that we are interested in is where the electronic and vibrational spectra of ionic species can be obtained by monitoring directly the energy loss of an ion in a fast (500-10000 eV) collision process. It is initially assumed that the excitation energy can be directly measured from the loss of translational energy of the fast ion species however energy may also be taken up by the target [2]. In addition the ion may be scattered through an angle which might affect the energy measurement by the analysing sector. We have designed a collision cell which allows the energy given to the target in a collision process to be measured in coincidence with the energy loss (gain) of the fast ion species. This modified collision cell is being fitted to an ultra high resolution energy loss tandem mass spectrometer [3]. The consequences of this design will be that the energy distribution imparted to the target during collision can be deconvoluted, either at a later stage or immediately by simultaneous energy analysis of the scattered target gas molecule. This will enable spectra free from target effects to be obtained reflecting the structure of the ion alone and will allow considerably higher spectral resolutions to be obtained. Reactions that are particularly suited to this technique are when the collision results in the target gas being ionized or gaining a charge by charge exchange or electron capture or if an electron is stripped off the fast ion or target species.

Figure 1 shows a schematic diagram of the collision cell and its location in the energy loss mass spectrometer. Several types of energy analysers could be chosen and used for energy analysis of the ionized target species. A cylindrical electrostatic analyser is shown, which is suitable for ions but for electrons a hemispherical analyser would be preferable or a simpler arrangement of a parallel plate analyser would also work effectively for both electrons and ions. The ion optics of the collision cell are a derivation of the electron source design of Pierce [4] and are shown in Figure 2 schematically, the cell was also modelled using the SIMION [5] programme which allowed some further optimisation. Figure 2 shows a plan view of the cell for a given set of potentials applied to the cell repeller and side beam shaping electrodes. The purposes of these side electrodes is to shape the potential distribution within the cell so that an equipotential is formed along the path (XX') of the ion beam. Thus any target molecules that are ionized (or electrons ejected) will be formed at a given potential and will be capable of being refocussed on the entrance slit of the auxiliary energy analyser. Use of coincidence counting techniques allows the energy imparted to the target to be measured and correlated to the energy loss of the fast ion. The method by which this is achieved is illustrated in Figure 3 whereby a delay is set between the arrival of the target species (at detector D1) and the fast ion species (at detector D2). The efficiency of coincidence counting is less efficient than that for a single channel and is the product of the efficiencies for detecting the target species multiplied by that for the fast ion species.

Figure 4 illustrates for charge stripping how the deconvolution of the energy spread imparted to the electron during the collision process can lead to a significant improvement to the charge stripping spectrum. However in this case it does not account for the energy given to the target so is only a partial solution. For reactions involving charge transfer then monitoring the ionized target together with the fast ion fully describes the partitioning of energy in the reaction. Currently although the energy resolution of the current instrument is ca. 0.015 eV for a 3000 eV ion beam, the achievable product ion energy resolution is generally around a factor 1.5 to 10 worse. This new collision cell design shows promise to solve this deficiency of Translational Energy Spectroscopy (TES).
Figure 1  TES Spectrometer and the Extraction Collision Cell.

Figure 2  Potential Distribution in the Collision cell.

Figure 3  Representation of the Coincidence Timing Circuit.

Figure 4  a) Charge stripping spectrum of Ar⁺ on NO.
           b) Computer simulated deconvoluted spectrum.

References
5  Obtained from D Dahl and J E Delmore, EG&G Idaho Falls, Idaho.
INTRODUCTION

There has been increasing interest in the use of metal ion attachment as a means for directing fragmentation of large molecules. Recent work by several groups has shown that specific interactions between metal ions and biopolymers are important in fragmentation processes. These studies have suggested that cation attachment may lower activation energies and enhance certain fragmentation processes. Recently, Lattimer and coworkers have reported the fragmentation of synthetic polymers with attached protons and sodium ions at relatively high energies (> 50 eV). They reported fragmentation processes that appeared to be both charge initiated and charge-remote. Lattimer's work suggested that specific metal ion dependent fragmentation processes might be observed. Energy resolved collision-induced dissociation (CID) at relatively low energies (0-200 eV) may prove useful in the elucidation of such processes. In this study we report the low-energy fragmentation of protonated and cationized poly(ethylene glycols). To determine the effect of the cation species, we have studied lithium, sodium and potassium adducts.

EXPERIMENTAL

Low-energy collision-induced dissociation tandem mass spectra were obtained on a Finnigan TSQ-70 (0-200 eV) or a ZAB-2FQ reverse geometry hybrid instrument (BEqQ; 0-500 eV). MIKE spectra of unimolecular decomposition products were obtained on the ZAB and were obtained by scanning the electric sector for decompositions between the magnet and ESA (FFR2). Unimolecular decompositions in FFR3 of the hybrid were obtained by mass-selecting a parent in the double focussing mode and scanning the quadrupole for decomposition products. Low-energy CID was carried out with Xe (at near single collision conditions) or Ar (both single or multiple collision conditions as indicated).

The polyethylene glycols (PEGs) used in these studies were obtained from Baker Chemical and had estimated number average molecular weights ($M_n$) of 400, 600 and 1000. Ions were formed by fast-atom bombardment of neat polyethylene glycols (PEGs), or the PEGs mixed with alkali halide salts. Parent ions were selected with unit resolution unless indicated otherwise.

RESULTS AND DISCUSSION

Unimolecular decomposition spectra and low-energy CID spectra of protonated PEGs formed by FAB show that there are two major ion series formed by dissociation. As proposed by Lattimer, these series could arise from a charge-initiated mechanism, as suggested in the scheme presented below. The unimolecular decomposition spectrum is very sensitive to the flight time. The B series of ions result from loss of (mx44)+18 and predominate at longer flight times. CID of the protonated species tends only to shift the B series to lower molecular weights.

![Chemical structure](image)

The results are considerably different with alkali ion adducts of the PEGs. Figure 1 shows the low-energy CID spectrum of [HO(CH₂CH₂O)₄H + Na]⁺. Two series of ions are observed with 10 eV collisions, corresponding to the scheme below. An unusual feature of the spectrum is the enhanced abundance of the ion corresponding to the neutral loss of 150 amu (HO(CH₂CH₂O)₄OH). This enhanced loss of 150 amu is observed with higher molecular weight PEGs in both unimolecular and CID spectra. This result suggests that specific ion-oligomer interactions may be involved in the fragmentation process, as this enhanced loss is not observed with protonated molecules.

Figure 2 shows the 150 eV CID spectrum (Xe collision gas) of the same Na adduct. The spectrum shows...
considerably more fragmentation. At least 4 additional types of fragmentation are observed, as depicted in the schemes below. The C series of ions has been identified by Lattimer et al. in previous studies, and they have postulated that it arises from a charge remote process involving 1,4 hydrogen elimination. The complementary ion (D) has the same mass as the B series above, however. The ion series labelled E, F and G all arise from heterolytic bond cleavages and most likely form distonic radical cations. They do not appear in the spectra of protonated PEGs. This suggests that the alkali ions lower the activation barriers for fragmentation, possibly through specific ion-PEG interactions. The fact that similar types of processes are observed with Li$^+$ and K$^+$ adducts supports the idea that ions lower the activation energies for these processes. It should be noted, however, that the preferential loss of 150 amu is not observed with Li$^+$ and K$^+$ adducts, suggesting that there are interactions that are specific to a particular species of ions as well.

Figure 1. 10 eV CID of [HO(CH$_2$CH$_2$O)$_{12}$H$^+$Na$^+$(Xe, near single collision)

Figure 2. 150 eV CID of [HO(CH$_2$CH$_2$O)$_{12}$H$^+$Na$^+$(Xe, near single collision)

A BEAM DYNAMICS STUDY OF THE ROLE OF INTERNAL AND KINETIC ENERGY ON
COLLISION-INDUCED DISSOCIATION OF LOW ENERGY IONS

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Recent studies of collision-induced dissociation (CID) of polyatomic ions at low collision energies have demonstrated that kinetic as well as internal energy of the ion play a very important role in determining the dynamics and mechanisms of collisional activation and subsequent dissociation [1-3]. Using a crossed supersonic beam tandem mass spectrometer, we have demonstrated backward and superelastic scattering processes, incomplete energy randomization after collisional activation as well as initial preparation by electron ionization and change from vibrational to electronic excitation of the ion on collision. These studies also provided a strong correlation of the CID behavior with the photoelectron spectrum of the molecule.

We have extended our CID studies to benzene ion whose photoelectron spectrum shows similar characteristics of large energy gaps between photoelectron bands as in acetone and nitromethane molecules. Also, many studies [4,5], on the fragmentation of the benzene ion have suggested that its dissociations to C_4H_4^+ and C_3H_3^+ occur from a different state than the dissociations to C_6H_6^+ and C_6H_4^+. An excited electronic state or an isomeric structure may be involved for these dissociation processes. However, a recent study by Baer et al. [6] showed that the dissociation of benzene ion proceeds from the ground state in agreement with the statistical theories. Another interesting feature in benzene ion dissociation process is the kinetic shift; thermochemical threshold for C_4H_4^+ is 13.85 eV, however, experimentally measured threshold is 14.30 eV.

Figures 1 and 2 show velocity contour maps for the dissociation of benzene ion to C_4H_4^+ at 69.5 and 10.1 eV collision energies, respectively. Intensity maximum for 69.5 eV CID is located in the forward scattering region at 7 deg. (CM) corresponding to the energy transfer of -7 eV. At 10.1 eV collision energy, however, the contour map shows the occurrence of a very complex CID process. Three intensity maxima in backward, forward and superelastic forward scattering regions are observed. As collision energy is reduced further, intensity peaks in backward and forward scattering regions are still present but only a tail extending to backward superelastic region is observed. It may be due to large energy requirement for this dissociation channel.

Integration of Figure 2 over all scattering angles gives the relative translational energy distribution (P(T) diagram) shows a very broad energy distribution with probability maxima corresponding to the energy transfer from kinetic to internal and internal to kinetic of -4.7 eV. This energy transfer from kinetic to internal energy corresponds to the excitation of ground state benzene ions to third electronic band. Similarly the excess kinetic energy gained by the dissociating benzene ion corresponds to the release of excess electronic energy via de-excitation of benzene ions in the seventh electronic band to the third electronic band. These latter ions have sufficient internal energy to dissociate from that surface. Further experiments are in progress to understand the complex dissociation mechanisms of benzene molecular ions to C_4H_4^+ and C_3H_3^+.

References:
Figure 1. Velocity contour map for the CID of benzene molecular ion to $C_4H_4^+$ with Ar at 69.5 eV collision energy.

$C_6H_6^+ + \text{Ar} \rightarrow C_4H_4^+ + C_2H_2 + \text{Ar}$

$E_{\text{CM}} = 69.5 \text{ eV}$

Figure 2. Velocity contour map for the CID of benzene molecular ion to $C_4H_4^+$ with He at 10.1 eV collision energy.

$C_6H_6^+ + \text{He} \rightarrow C_4H_4^+ + C_2H_2 + \text{He}$ (10.1 eV)

Figure 3. Relative translational energy distribution of $C_4H_4^+$ ions at 10.1 eV collisional energy.
Examination of Ortho Effects in the Collisionally Activated Dissociation of Closed Shell Disubstituted Aromatic Ions. Tracy Donovan and Jennifer Brodbelt, University of Texas, Austin, TX 78712-1167.

Functional group interactions classified as "ortho effects" have been characterized for many disubstituted open shell aromatic ions. These effects are manifested as specific dissociation pathways that are observed for ortho substituted aromatic compounds that are not observed for the other isomers. Such effects have been reported for a variety of aromatic compounds, including methoxybenzaldehyde, acetophenones, nitrophenol, phenylacetylenes, phenyl sulfides, benzoic acids and many more complex aromatic species. The importance of ortho effects in influencing the reactive and dissociative behavior of closed shell aromatic ions has not been extensively examined.

We have studied the reactive and dissociative behaviors of the ortho, meta and para isomers of five disubstituted aromatic compounds containing various combinations of electron-releasing and electron-withdrawing substituents: methoxyacetophenone, hydroxyacetophenone, anisaldehyde, methoxy phenol, and hydroxybenzaldehyde. The results for methoxyacetophenone, anisaldehyde, and methoxyphenol are reported. The two main objectives were to compare the dissociative behavior of various closed shell ions generated via ion/molecule reactions of the p-, m-, and o-isomers, and to elucidate the mechanisms for the unusual ortho effects. The ions of interest were generated by proton transfer from dimethyl ether, or by methylene substitution or methyl addition from ion/molecule reactions with dimethyl ether or ethylene oxide. The structures and dissociative pathways of the ions were characterized by low-energy collisionally activated dissociation in a quadrupole ion trap and a triple quadrupole mass spectrometer and by comparison to the behavior of model ions. The CAD spectra of related monosubstituted aromatic ions were also acquired. These latter spectra served as a basis to determine which of the substituents in the disubstituted aromatics had the dominant role in directing reactive and dissociative pathways.

**Methoxyacetophenone.** The methoxyacetophenone isomers contain an electron-withdrawing acetyl substituent and a moderately electron-releasing methoxy substituent. The CAD spectra of the open shell radical cations, M+-, of o-, m-, and p-methoxyacetophenone generated by electron ionization are qualitatively similar, all showing extensive loss of methyl radical and formation of a variety of aromatic-type ions (C6H5*, C7H7+). However, the CAD spectra of isomeric closed-shell (M + H)+ methoxyacetophenone ions show several striking differences (Figure 1). The protonated meta and para isomers both dissociate via loss of methane and via formation of acetyl cation (formation of m/z 135 and formation of m/z 43 respectively), which is more similar to the behavior of protonated acetophenone than protonated anisole. These processes are not the most significant ones observed for the ortho isomer. Instead, the ortho isomer produces m/z 133 via dehydration, and m/z 105, the most abundant fragment ion.

The unusual ion of nominal mass-to-charge 105 in the CAD spectrum of protonated o-methoxyacetophenone could presumably be C6H5H+ (an alkyl aromatic ion) or C7H5O+ (an aromatic carbonyl ion). Sequential collisional activation experiments in the quadrupole ion trap were performed to determine the structural composition and mechanism of formation of the ion at m/z 105. First the protonated o-methoxyacetophenone ion at m/z 151 was isolated and collisionally activated. Using a second stage of selected ion isolation, the daughter at m/z 105 was chosen and all other ions were ejected from the trap. Then m/z 105 was excited at its resonant frequency to induce dissociation. The major dissociation routes are by dehydrogenation (formation of m/z 103) and loss of 26 amu (formation of m/z 79).

The identity of the m/z 105 ion was elucidated by comparison of CAD spectra of model ions generated from o-xylene, (M - H)+ or C6H5+, and benzaldehyde, (M - H)+ or C7H5O+. The o-xylene ion dissociates predominantly via loss of H2 and C2H2, whereas the benzaldehyde ion dissociates via loss of CO only. The CAD spectrum of the o-xylene ion closely resembles that observed for the m/z 105 generated from o-methoxyacetophenone, and therefore the composition of this ion is assigned as C6H5H+. This composition is somewhat surprising because it must involve extensive rearrangement of the protonated o-methoxyacetophenone in order to transfer both methyl groups to the ring. Sequential activation experiments show that it is likely that the ion of m/z 105 results from sequential dehydration then decarbonylation of the ion of m/z 151, and that the ion of m/z 133 is a direct precursor to m/z 105.
A mechanism for this process is suggested in Scheme 1. Under the near equilibrium conditions during ionization, the thermochemically favored \((M + H)^+\) ion is formed, with the proton localized at the most basic site (the carbonyl oxygen). Upon collisional activation, the proton re-locates at the ortho-methoxy substituent and the methyl group transfers to the aromatic ring. This is followed by elimination of H\(_2\)O via a four-membered-ring transition state. Subsequently, the positively-charged aromatic ring is stabilized by another methyl transfer from the acetyl substituent, and then CO is expelled. The resulting product is a dialkyl aromatic ion.

Ortho effects play an important role in the collisionally activated dissociation of closed shell disubstituted aromatic ions. These effects are more dramatic in the dissociation of ions formed from ion/molecule reactions than in the dissociation of isomeric open shell radical cations generated by electron ionization. Moreover, the dissociative behavior of adduct ions, such as \((M + 15)^+\) and \((M + 13)^+\) ions generated by ion/molecule reactions with dimethyl ether or ethylene oxide, may demonstrate pronounced ortho effects even when such effects are not observed in the CAD spectra of the simple protonated ions. The dissociative processes of the disubstituted meta and para aromatic ions are often analogous to ones observed for monosubstituted aromatic ions. In general, for disubstituted aromatics containing both electron-withdrawing and electron-releasing substituents, the dissociative behavior of the \((M + H)^+\) and \((M + 15)^+\) ions of the meta and para isomers reflect the behavior observed for the corresponding monosubstituted aromatic ions containing electron-withdrawing substituents. Conversely, the \((M + 13)^+\) ions of those meta and para isomers dissociate via pathways observed for the monosubstituted aromatic ions containing electron-releasing substituents, such as methoxy or hydroxyl groups. Many of the dissociative mechanisms of the ortho isomers can be rationalized as involving proton transfer or cyclization between the adjacent functional groups. Because of the ortho effects, these latter mechanisms do not reflect the behavior observed for the monosubstituted aromatic ions.

![Figure 1. CAD Spectra of Protonated o-, m and p-Methoxycacetophenone (m/z 161) Acquired with a Quadrupole Ion Trap Mass Spectrometer](image)

![Scheme 1. Proposed Mechanism for Loss of H\(_2\)O and CO from Protonated o-Methoxyacetophenone](image)
Collisionally activated dissociation (CAD) is one of the most popular techniques in tandem mass spectrometry. However, the internal energy that can be deposited in an ion upon collision with a stationary target is limited by the ion center-of-mass kinetic energy \( T_{cm} \) which shows a hyperbolic decay with increasing ion mass. A rigorous treatment of the two-body collision kinematics based on König's theorem yields a three-term expression for \( T_{cm} \), where \( m_1, T_1, p_1, \) and \( m_2, T_2, p_2 \) are the mass, laboratory kinetic energy, and momentum vectors for the ion and the target, respectively. For stationary or thermal energy targets \( (T_2 = 0.038 \text{ eV}) \) the second term becomes negligible, while the third term only broadens the internal energy distribution. By contrast, for high kinetic energy targets, e.g. fast atoms produced by a FAB source, or keV ions or electrons produced by ion or electron guns, the collision kinematics depends on the particle masses and relative velocities.

According to (1) \( T_{cm} \) of slow (1-100 eV) analyte ions colliding with a fast target increases with the ion mass to reach the \( T_2 \) limit (Figure 1).

![Figure 1. Center-of-mass kinetic energy in collisions of 6 keV \(^{131}\text{Xe} \) atoms with 10 eV ions of mass \( m_1 \).](image-url)
This contrasts the decrease of $T_{\text{CM}}$ with increasing ion mass in collisions with stationary targets. In fast (keV) ion-fast target collisions $T_{\text{CM}}$ depends on the encounter orientation (head-on, perpendicular or chasing) due to a non-negligible momentum term in (1). However, at very high masses ($m > 100$ kDa) $T_{\text{CM}}$ converges to $T_2$, too (Figure 2). These features could make collisions with fast targets an attractive alternative to other ion-activation techniques. The technical aspects of the fast ion-fast target collisions will be discussed.

Figure 2. Center-of-mass kinetic energy in collisions of 6 keV $^{131}$Xe atoms with 8 keV ions of mass $m_1$. 
INTRODUCTION

Tandem mass spectrometry is a powerful tool for obtaining amino acid sequence information for peptide ions. Collisionally activated dissociation (CAD) is the most widely applied tool for peptide tandem mass spectrometry. Photodissociation (PD) has been studied by several investigators (1-6) as an alternate method for dissociating peptide ions. PD offers the advantage that the energy deposited into the parent ion is independent of the mass of the precursor, in contrast to CAD, in which energy deposited decreases with increasing precursor mass. However, efficient PD requires that the excitation wavelength is strongly absorbed by the peptide. The solution spectra of most peptides show a strong absorbance between 190 nm and 225 nm. Most previous studies of peptide PD have used 193 nm light from an excimer laser (1-5). In this study, we compare PD and CAD of a number of small peptides.

EXPERIMENTAL

These experiments were performed in a Fourier transform mass spectrometer equipped with a 4.4 cubic centimeter analyzer cell, IonSpec Omega data system and electromagnet operating at 1 Tesla. Peptide ions were formed by laser desorption/ionization of the neat sample. Since a single laser is utilized for these studies, 193 nm light from the ArF line of an excimer laser (Questek, Model 2110) is used for desorption/ionization as well as PD. The irradiance used for laser desorption and for PD is ca. 10^7 watts/cm^2. The peptide samples were prepared by electrospraying 100 - 200 microliters of a 10^{-3} M solution of the sample in methanol on a 75 nm thick Au/Pd (40:60) coated Macor probe. All CAD experiments were conducted in the low energy regime with Ar as the collision gas. The PD experiments used from 10-99 laser pulses.

RESULTS

The generation of sequence specific ions by PD and CAD have been studied for all the isomers of VPL and FGGF. The data obtained by each ion activation method provides sufficient fragmentation information to determine the amino acid sequence. Interestingly, the FGGF isomer requires approximately twice the number of PD pulses to obtain the same relative amount of fragmentation as the other two isomers. CAD experiments show that all three isomers fragment with the same efficiency. These results suggest that the FGGF isomer does not absorb energy as efficiently as its isomers. Condensed phase UV-VIS spectra have been measured, and appear identical for each isomer typically showing a broad absorption envelope from 190 - 230 nm. Molar absorbivities in solution are the same for the three isomers, within experimental error. In the gas phase the absorption bands are known to be more discrete thereby exposing holes in the absorption spectrum which may overlap with the PD wavelength. We have applied PD and CAD to tetra- and pentapeptides, and obtained adequate information to identify the amino acid sequence. We find that higher mass fragment ions tend to be produced more abundantly by CAD while lower mass ions tend to dominate the PD spectra.

We have performed matrix assisted laser desorption at 248 nm (KrF) for moderately sized peptides, gramicidin S and gramicidin D. Because of the mass of these peptides, the CAD technique does not impart enough energy for fragmentation in our instrument. PD at the wavelength of the desorption laser (248 nm) did not yield any daughter ions, as the peptides absorb weakly at this wavelength. Further studies of these middle sized molecules will require separate lasers for desorption and photodissociation. The application of the KrF line (222 nm) will be studied, as this wavelength is close to \lambda_{max} for most peptides.
Figure 1. Comparison of PD and CAD mass spectra for a tripeptide, tetrapeptide, and pentapeptide.
KINETICS and MECHANISM of the COLLISIONALLY-ACTIVATED DISSOCIATION of the ACETONE CATION

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Used NIST kinetics-based measurement protocol\(^1\) to study kinetics, energetics, and mechanism of collisionally-activated dissociation (CAD) of acetone cation (m/z 68) under single-collision conditions (Ar target)\(^8\) in NIST QqQ tandem mass spectrometry (MS/MS) instrument.\(^3\)

- Measured CAD breakdown curves; viz., the dynamically-correct (absolute), Instrument-Independent branching ratios (product distributions) of CAD fragment ions as function of center-of-mass collision energy \(E_{cm} = 1-60\) eV (Table 1 in ref. 2).
- Major fragment ions are MeCO\(^+\) (m/z 43; branching ratios of 0.96-0.60) and Me\(^+\) (m/z 16; branching ratios of 0.02-0.26) — see Table 1 and Fig. 1 in ref. 2.
- Ketene cation (m/z 42; branching ratios of 0.02-0.06) is minor fragment (Table 1 and Fig. 3 in ref. 2).
- For \(E_{cm} = 1-60\) eV, absolute total cross sections were 24 to 36 \(\text{Å}^2\).

Estimated average internal energy \(E_{int}\) of collisionally-activated acetone cation by normalization of our CAD data relative to corresponding PEPICO (photoelectron-photolysis-coincidence) breakdown graphs from refs. 4 and 6, and to internal energy deposition functions \(P(E)\) from ref. 6.

- \(E_{int}/E_{cm}\) vs. \(E_{cm}\) dependence observed in NIST QqQ instrument for 58—>43\(^+\) (thermodynamic threshold energy \(E_{thr} = 4.24\) eV) in good agreement with that derived from Fig. 8-12 of ref. 6 for 68—>43\(^+\) (\(E_{thr} = 0.82\) eV), with the plots shifted relative to each other by the difference in the respective \(E_{thr}\) (Table 2 and Fig. 2 in ref. 2).

Concordance of CAD and PEPICO data in Figs. 1 and 2 of ref. 2 is consistent with knowledge (from molecular beam studies\(^7\)\(^9\)) that: (a) CAD excitation mechanism follows electronic excitation ladder for acetone and nitromethane cations; (b) these cations have bands of electronically-isolated states which do not communicate efficiently with ground state (cf. photoelectron spectrum (PES) in ref. 10); (c) CAD of acetone and nitromethane cations violates fundamental principle of 'strong version' of quasi-equilibrium theory (QET) of mass spectra, which requires strong coupling of excited states to the ground state;\(^5\) and (d) there's an apparent correlation between extraordinarily efficient interconversion of electronic and translational energy effected by low-energy ion-neutral collisions\(^5\)\(^4\), and large gap (or 'window') in corresponding PES of more than 1 eV between the ground and excited states (first and higher ionization bands) (cf. PES of nitromethane and acetone\(^10\)).

- Indicates that dissociation occurs primarily from same electronic states in both CAD and PEPICO experiments.
- Indicates correspondence between PEPICO and CAD breakdown curves for systems which have isolated electronic states (as does the acetone cation).\(^5\)\(^7\)\(^9\)
- Suggests collisional activation in such non-QET systems may correspond to scattering angles which emphasize optically-allowed transitions accessed by photolization.

Rate of production vs. \(E_{int}\) of each CAD fragment ion is consistent with a reaction mechanism wherein \(E_{int}\) for each source reaction coincides with an ionization band in the photoelectron spectrum (PES) of MeCO\(^+\) (cf. ref. 10).\(^4\) For example, our CAD breakdown curves for 58—>43\(^+\) and 58—>15\(^+\) show complex, complementary energy dependences for production of MeCO\(^+\) and Me\(^+\). This is consistent with competition between three fast, primary (direct) reactions (viz., (1)-(3)), each of which exhibits non-QET behavior, and each of which opens sequentially at its respective \(E_{int}\).

\[
\begin{align*}
\text{MeCO}^+ & \rightarrow \text{MeCO}^+ + \text{Me} (X ^1A'') & \Delta H = 0.82 \text{ eV} & (1) \\
& \rightarrow \text{Me}^+ + \text{Me} + \text{CO} & \Delta H = 4.24 \text{ eV} & (2) \\
& \rightarrow \text{MeCO}^+ + \text{Me} (B, 1 ^1A') & \Delta H = 6.56 \text{ eV} & (3)
\end{align*}
\]
This proposed mechanism is consistent with refs. 6 and 7 if correspondence between the CAD and PEPICO data were due to having the endoergic reactions (2) and (3) proceed by pumping ground state ions to excited electronic states of Me$_2$CO$^+$, so that CAD occurs predominantly from these excited electronic states.

- For reaction (2), collisional activation of Me$_2$CO$^+$ involves direct electronic excitation from ground state of Me$_2$CO$^+$ (a) into C state of Me$_2$CO$^+$ (4th ionization band$^{10}$) and (b) into E state of Me$_2$CO$^+$ (6th ionization band$^{10}$).
- $\Delta E_{th}$ for (2) coincides with 4th ionization band$^{10}$.

- For reaction (3), CAD involves direct electronic excitation into G state of Me$_2$CO$^+$ (8th ionization band).
- $\Delta E_{th}$ for (3) coincides with 8th ionization band$^{10}$.

Other minor fragments observed are consistent with production via Me-C=O-Me (I)

\[
\begin{align*}
\text{Me}_2\text{CO}^+ & \rightarrow \text{MeCH}_2^+ + \text{HCO}^- & \Delta H = 2.36 \text{ eV} \quad (4) \\
& \rightarrow \text{HCO}^+ + \text{MeCH}_2^- & \Delta H = 2.32 \text{ eV} \quad (5) \\
& \rightarrow \text{CH}_2\text{H}^+ + \text{H}_2\text{CO} & \Delta H = 2.47 \text{ eV} \quad (6) \\
& \rightarrow \text{CH}_2\text{H}^+ + \text{MeO}^- & \Delta H = 4.24 \text{ eV} \quad (7) \\
& \rightarrow \text{CH}_2\text{H}^+ + \text{MeOH} & \Delta H = 4.22 \text{ eV} \quad (8) \\
& \rightarrow \text{CH}_2\text{H}^+ + \text{MeO}^+ & \Delta H = 4.01 \text{ eV} \quad (9) \\
& \rightarrow \text{CH}_2^+ + \text{MeCHO} & \Delta H = 5.18 \text{ eV} \quad (10) \\
& \rightarrow \text{H}_2\text{C} = \text{CH} = \text{O}^+ + \text{H} + \text{Me} & \Delta H = 5.43 \text{ eV} \quad (11) \\
& \rightarrow \text{CO}^+ + \text{H} + \text{CH}_2\text{H}^+ & \Delta H = 5.96 \text{ eV} \quad (12)
\end{align*}
\]

- $\Delta E_{th}$ for reactions (4)-(6) coincide with onset of 2nd ionization band$^{10}$ (A state).
- $\Delta E_{th}$ for reactions (7)-(9) coincide with onset of 4th ionization band$^{10}$ (C state).
- $\Delta E_{th}$ for reactions (10)-(12) coincide with onset of 6th ionization band$^{10}$ (E state).

This work further validates that NIST kinetics-based measurement protocol can provide dynamically-correct (i.e., instrument-independent) representation of CAD (and other ion-neutral interactions) under single-collision conditions in MS/MS instruments which use rf-only multipole collision cells.$^{10}$

REFERENCES

A well demonstrated advantage of neutralization-reionization mass spectrometry (NRMS) is to obtain isomerically characteristic mass spectra from hydrocarbons and other ions whose isomerizations are facile relative to their dissociations. Vertical charge exchange moves the analyte ion onto the neutral potential energy surface, where isomerization barriers can be higher relative to those for fragmentation. This NRMS capability has now been applied to the MS determination of deuterium position in hydrocarbons, specifically toluene and styrene, whose mass spectra show a high degree of hydrogen scrambling prior to ionic fragmentation.

The mass spectra of α- and ar-α-toluene from electron ionization and from collisionally activated dissociation are nearly identical because of the rapid equilibration of various isomeric C₆H₅· structures before dissociation. Neutralization of the toluene ions with metal vapors of low ionization potential (IP) yields toluene molecules whose internal energy content reflects the difference between the electron affinity of the toluene ion and the metal IP. As shown in the Figure, this should yield toluene with increasing internal energy as the metal vapor is changed from Hg to Na, K, and Cs. Because the rearrangement reaction is entropically unfavorable, increasing the internal energy of the toluene molecules decreases the degree of isomerization before dissociation, as shown by the increasing relative favorability for the loss of D vs. H from toluene-α. Angle-resolved NRMS, described in a separate abstract, should reduce further the scrambling accompanying Cs neutralization.

The isomerization of styrene is even more facile, with NRMS spectra using even Cs neutralization showing almost complete isotopic scrambling accompanying H/D loss from styrene-α and -γ. Although there is little difference between Na, K, or Cs neutralization, the higher energy losses of C₂H₂ and C₂H₃ are not accompanied by complete scrambling; for the d₁ compounds the losses are: C₂H₂ 32%, C₂HD 37%, and C₂D₂ 29%; C₂H₃ 37%, C₂H₂D + C₂HD 34%, and C₂D₃ 29%. Actually, the scrambling is not complete for these losses using CAD, but rearrangement is reduced by a further ~15% by NR for both C₂H₂ and C₂H₃ losses. Stepwise isomerization to cyclooctatetraene explains the favored losses of C₂H₂/C₂D₂ and C₂H₃/C₂D₃ from the d₁ and d₁ precursors.

Linked scanning of the final electrostatic and magnetic analyzers of the four-sector instrument made possible complete resolution of these peaks in the CAD and NR spectra. The m/z 80 and 82 peaks from the d₁ and d₁, precursors actually represent mixtures of C₂H₂D₃, C₂H₂D₂, and C₂H₂D, and C₂H₂D, respectively; the proportions of these in the respective CAD spectra were determined in an MS/MS/MS experiment in the four-sector EBEB instrument by dissociating these ions in the fourth field-free region followed by mass analysis in magnet-II.
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CAD, $O_2$ (50% T)

NR, Hg (70% T), $O_2$ (70% T)

NR, Na (275° C), $O_2$ (70% T)

NR, K (190° C), $O_2$ (70% T)

NR, Cs (134° C), $O_2$ (70% T)

M/Z 190 191 192 193 194 195 196 197

IP 95/96 % scr

0.60 100
0.88 89
0.69 98
10.4 0.85 ~99
5.1 0.66 <77*
4.3 0.49 <57*
3.9 0.41 <48*
USE OF A CONTINUOUS IONIZATION SOURCE WITH A DOUBLE FOCUSING
MAGNETIC SECTOR (EB)/REFLECTRON TIME-OF-FLIGHT
MASS SPECTROMETER
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The standard mode of operation for time-of-flight instruments is to form ions by using a pulsed
ionization source, e.g., pulsed Cs+ ion source, pulsed laser (for multiphoton ionization or desorption), and,
assuming the ions are formed with a narrow distribution of kinetic energies, the mass resolution of the
time-of-flight measurement is limited by the temporal characteristics of the ionization pulse. Thus,
ionization pulse durations of 1-3 nanoseconds are required for high mass resolution measurements.
Although pulse widths of 1-10 nanosecond can be obtained by rastering a convention Cs+ ion gun across
a small slit, this mode of operation requires summing a large number of events (10^6 or greater) to obtain
a complete mass spectrum. The requirement of a large number of ionization events presents a major
obstacle to performing tandem mass spectrometry by using a pulsed ion source. For example, the
efficiency of collision-induced dissociation is quite low ranging from a few per cent to 0.1-0.5% for large
molecules. If we assume a collision-induced dissociation efficiency of 0.1%, then 10^6 primary ions yields
10 fragment ions. Because the number of reaction channels available to the collisionally activated ion
is quite large (say 10-15 structurally significant fragment ions are typical for relatively small peptides), it
may be necessary to accumulate data from 10^6-10^7 ionization events in order to collect a statistically
significant data set. If the repetition rate of the ionization source is 10 kHz a data set of 10^6 events can
be obtained in 100 seconds. However, as the ion yield or collision-induced dissociation efficiency
decreases, as is observed for increasing molecular weight, the number of events required to collect a
statistically significant data set also increases.

An alternative approach to performing measurements with a pulsed ion beam is to form large
numbers of ions per ionization pulse and collect the data by using a transient recorder. This is a common
mode of operation for matrix-assisted laser desorption where the yield of ions per laser pulse is high, e.g.,
10-100 ions are formed for each laser pulse. The limitation of this experiment is the high rate of sample
consumption and the inherent loss in sensitivity over single ion counting experiments. The sample
consumption rate does not effect acquisition of normal mass spectral data but this does pose a problem.
for signal-averaging. In addition, because the efficiency of the activation/dissociation process is low the transient recorder mode is less effective for acquisition of collision-induced dissociation and/or photodissociation data.

This paper describes a novel method of data acquisition for a magnetic sector(EB)/ reflectron time-of-flight mass spectrometer, which uses a continuous source. The method is based on the detection of a neutral to start the time-of-flight clock for the ion. Shown in Figure 1 is the metastable spectrum of Gly-Pro-Gly-Gly obtained with this method.

**Metastable Spectrum of Gly-Pro-Gly-Gly**

![Metastable Spectrum of Gly-Pro-Gly-Gly](image)

Figure 1.
THEORETICAL INVESTIGATION OF THE STRUCTURES OF C5-C8 ALKANES AND ALKYL CATIONS
IN COLLISION ACTIVATION

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Computer-automated interpretation of mass spectra involves the correlation of an experimental mass spectrum to a chemical structure. In practice, MS interpretation programs generate a list of most probable structures which, in order to provide useful information, has to be evaluated according to the degree of consistency between the proposed structure and the unknown spectrum. The consistency test requires the prediction of appropriate spectral features for each candidate structure, and further classification according to best match results obtained by comparison with the experimental mass spectrum of the unknown structure. Rule-based mass spectrum prediction methods have been developed for candidate structure ranking, but those methods are of limited success and are based on ion fragmentation plausibilities and not on actual ion intensities. Fragmentation theories based on calculated charge densities and bond orders have been developed, which in some instances can successfully deduce the most likely fragmentation pattern of mass spectra. In a previous study (1), such an approach based on a simple forced oscillator model has been evaluated for the generation of synthetic tandem mass spectra, to be used for classification of the candidate structures proposed by an MS interpretation program.

Although the model was found capable of correlating structures to activation spectra its application involved knowledge of force constants and charge distributions evaluated from the neutral molecules. However, upon ionization those values can change and a better approximation is required in order to provide more reliable force constants and charge distributions for ionic structures. Therefore, the objective of the present work has been to apply MO quantum methods to representative ionic structures in order to obtain typical structural and spectroscopic data which will improve the performance of the model, and at the same time, serve as a test for the validity of the assumptions.

Linear alkanes have been chosen as the first representative class of compounds, due to the structural simplicity of their ions, and the relatively simple fragmentation patterns in their CA spectra. Theoretical investigation involved the use of the MINDO/3, MNDO, and AM1 hamiltonians of the MOPAC (2) semi-empirical quantum program, and of a density functional quantum method (LCGTO-DF) (3). CA spectra were obtained by high energy (8 KeV) collisions with helium in a floated collision cell (1 KeV) at a pressure consistent with single-collision conditions (=10% beam attenuation). Under the employed experimental conditions, the CA spectra are considered to represent characteristic information about the structure of the dissociating ionic species. The most abundant ion in the spectra of the open-shell alkanes is the \([M-29]^+\) ion formed by the preferential loss of \(C_2\text{H}_5\). The loss of 15 daltons (CH3) is small for heptane and octane but higher for hexane and pentane. The alkyl cations show a more complex behavior compared to the alkane chains with \(C_5\), \(C_7\), and \(C_9\) yielding the \([M-42]^+\) as the most abundant ion, and \(C_5\) the \([M-28]^+\). Unfloated collision cell experiments for the hexyl closed-shell cation have shown the \([M-26]^+\) as the most intense ion peak in the MIKES/CA spectrum. Floating of the collision cell separates ions formed by natural and collisionally activated processes and reduces mass discrimination effects.

Semi-empirical methods were preferred initially because the calculation time is considerably shorter compared to \textit{ab initio} quantum methods. It was, however, found that MNDO yielded carbon-carbon bonds which were rather questionably long (=2Å). Thus, the other MOPAC hamiltonians MINDO/3 and AM1 were used in order to compare the methods and draw further conclusions on the ionic structures. A force calculation was conducted without any symmetry constraint for full optimization of the ion geometry. Tables 1 and 2 give heats of formation for the alkane and alkyl structures obtained by the various semi-empirical methods, and errors as compared to experimental data. AM1 was found to produce the most accurate \(\Delta H_f\) values, however, similarly to MNDO, AM1 calculated rather long C-C bond lengths, and asymmetric structures for the ions (TABLE 3). MINDO/3 produced reasonable bond lengths and retained the symmetry. It is interesting to note the correlation of the longer C-C bonds of the optimized structures with the experimental data. A similar correlation appears to hold true for the closed-shell cations, however, a clear conclusion cannot be drawn from the obtained results. It has been necessary, therefore, to resort to a higher level of calculations, in order to obtain a better degree of confidence in the data of the ion structures.

The geometry of the neutral \(C_5\text{H}_{12}\) molecule and two states (I and II) of the \(C_5\text{H}_{12}^+\) cation has been optimized with the LCGTO-DF method (3). The population analysis of the molecular orbitals of \(C_5\text{H}_{12}\) shows that the CC(middle) bonding orbitals lie closer (0.3 and 0.4 eV) to the highest occupied molecular orbital (HOMO) than the CC(end) bonding orbitals (1.1 and 2.0 eV), indicating that ionization from the
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The former is preferred. The two ionic states of \( \text{C}_5\text{H}_{12}^+ \) (I and II) correspond to ionization from the HOMO (CH-bonding) and the HOMO-1 (CC(middle)-bonding) levels respectively. The structure \( \text{C}_5\text{H}_{12}^+ \) (II) (after geometry relaxation) is more stable with respect to \( \text{C}_5\text{H}_{12}^+ \) (I) by about 0.3 eV. Table 4 shows that the CC(middle) bond length (R2) is longer than the CC(end) distance (R1) for the (II) state. The same trend is observed for the corresponding bond orders (0.9 and 1.1 for the CC(middle) and CC(end) respectively) in agreement with the experimental results.

Structural and spectroscopic data of ionic structures, in this study, were found to differ depending on the method employed for the calculations. Although, some methods yielded acceptable thermodynamic data, they generated questionable geometries. Therefore, caution is required for acceptance of a given method, and comparison of values calculated by the various methods is necessary in order to obtain chemically reliable data which are to be used for the prediction of fragmentation patterns of ionic species.

REFERENCES
(2) J.J.P. Stewart, QCPE Bull., 2, 133 (1985)

\[ \text{C}_5\text{H}_{12}^+ \] (I)
\[ \text{C}_5\text{H}_{12}^+ \] (II)

**TABLE 1. HEATS OF FORMATION OF THE C5-C8 ALKANE RADICAL CATIONS CALCULATED WITH MINDO/3, MNDO, AND AM1**
<table>
<thead>
<tr>
<th>CATION</th>
<th>MINDO/3</th>
<th>MNDO</th>
<th>AM1</th>
<th>EXP.</th>
<th>ΔHf</th>
<th>Error</th>
<th>ΔHf</th>
<th>Error</th>
<th>ΔHf</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-( \text{C}<em>7\text{H}</em>{13}^+ )</td>
<td>194</td>
<td>-10</td>
<td>221</td>
<td>+17</td>
<td>206</td>
<td>+2</td>
<td>204</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-( \text{C}<em>8\text{H}</em>{14}^+ )</td>
<td>185</td>
<td>-9</td>
<td>217</td>
<td>+23</td>
<td>199</td>
<td>+5</td>
<td>194</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-( \text{C}<em>9\text{H}</em>{15}^+ )</td>
<td>177</td>
<td>-7</td>
<td>212</td>
<td>+28</td>
<td>192</td>
<td>+8</td>
<td>184</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-( \text{C}<em>{10}\text{H}</em>{16}^+ )</td>
<td>169</td>
<td>-8</td>
<td>207</td>
<td>+30</td>
<td>185</td>
<td>+8</td>
<td>(177)</td>
<td></td>
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</table>

**TABLE 2. HEATS OF FORMATION OF THE C5-C7 ALKYL CATIONS CALCULATED WITH MINDO/3, MNDO, AND AM1.**
<table>
<thead>
<tr>
<th>CATION</th>
<th>MINDO/3</th>
<th>MNDO</th>
<th>AM1</th>
<th>EXP.*</th>
<th>ΔHf</th>
<th>Error</th>
<th>ΔHf</th>
<th>Error</th>
<th>ΔHf</th>
<th>Error</th>
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</thead>
<tbody>
<tr>
<td>n-( \text{C}<em>6\text{H}</em>{12}^+ )</td>
<td>195</td>
<td>-13</td>
<td>222</td>
<td>+13</td>
<td>206</td>
<td>+2</td>
<td>204</td>
<td></td>
<td></td>
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<tr>
<td>n-( \text{C}<em>7\text{H}</em>{13}^+ )</td>
<td>186</td>
<td>-11</td>
<td>218</td>
<td>+15</td>
<td>199</td>
<td>+5</td>
<td>194</td>
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<tr>
<td>n-( \text{C}<em>8\text{H}</em>{14}^+ )</td>
<td>178</td>
<td>-9</td>
<td>214</td>
<td>+24</td>
<td>192</td>
<td>+8</td>
<td>184</td>
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<td></td>
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<tr>
<td>n-( \text{C}<em>9\text{H}</em>{15}^+ )</td>
<td>170</td>
<td>-7</td>
<td>211</td>
<td>+28</td>
<td>188</td>
<td>+8</td>
<td>(177)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**TABLE 3. C-C BOND LENGTHS (Å) OF THE n-\( \text{C}_5\text{H}_{12}^+ \) RADICAL CATION CALCULATED WITH MINDO/3, MNDO, and AM1.**
<table>
<thead>
<tr>
<th>BOND</th>
<th>MINDO/3</th>
<th>MNDO</th>
<th>AM1</th>
</tr>
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<tbody>
<tr>
<td>1 C1C2</td>
<td>1.50</td>
<td>1.49</td>
<td>1.45</td>
</tr>
<tr>
<td>2 C2C3</td>
<td>1.54</td>
<td>1.97</td>
<td>2.08</td>
</tr>
<tr>
<td>3 C3C4</td>
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<td>1.50</td>
<td>1.46</td>
</tr>
<tr>
<td>4 C4C5</td>
<td>1.50</td>
<td>1.55</td>
<td>1.53</td>
</tr>
</tbody>
</table>

**TABLE 4. EQUILIBRIUM C-C BOND LENGTHS (Å) FOR \( \text{C}_5\text{H}_{12}^+ \) AND TWO STATES OF \( \text{C}_5\text{H}_{12}^+ \) OBTAINED BY THE LC-GTO-METHOD.**
<table>
<thead>
<tr>
<th>Bond</th>
<th>( \text{C}<em>5\text{H}</em>{12}^+ )</th>
<th>( \text{C}<em>5\text{H}</em>{12}^+ ) (I)</th>
<th>( \text{C}<em>5\text{H}</em>{12}^+ ) (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>1.51</td>
<td>1.49</td>
<td>1.51</td>
</tr>
<tr>
<td>R2</td>
<td>1.51</td>
<td>1.48</td>
<td>1.56</td>
</tr>
<tr>
<td>R3</td>
<td>1.12</td>
<td>1.14</td>
<td>1.11</td>
</tr>
<tr>
<td>R4</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>r4</td>
<td>1.11</td>
<td>1.11</td>
<td>1.13</td>
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</table>
CURIOUS KINETICS IN FRAGMENTATIONS OF N,N-DIMETHYLFORMAMIDE RADICAL CATIONS

Qinghong Ann and Jeanette Adams, Department of Chemistry, Emory University, Atlanta, GA 30322.

The neutral amide group has been a subject of many investigations because of its role as the peptide bond. Amides are fundamentally interesting because their lowest energy forms (planar) contain four delocalized \( \pi \) electrons in three \( \pi \) orbitals, analogous to the allyl anion [1]. \( N,N \)-Dimethylformamide (DMF) is particularly interesting because the HOMO of its planar form is the antisymmetric \( \pi \)-nonbonding orbital, which is followed in energy by the nonbonding oxygen lone pair [2]. Rotation about the C-N bond disrupts \( \pi \) delocalization and alters the orbital energies so that the HOMO now becomes the nitrogen lone pair orbital, which is of higher energy than the \( \pi \)-nonbonding orbital in the conjugated form [3].

Studies of gas-phase amide radical cations have primarily involved ion source decompositions of acetamide homologues [4]. Because there have been no studies of the formamide homologues, and because we are interested in the chemistry of the peptide bond [5], we began this study. All mass spectra shown here were acquired by using a VG 70-S, forward geometry mass spectrometer. Metastable ion and CID occurred in the first field-free region, and CID spectra were acquired at 50% main beam reduction with He. B/E scans were used to record the field-free region reactions.

Figure 1 shows 70-eV ion source (A, D), metastable ion (B, E), and CID (C, F) spectra of DMF and DMF-\( d_1 \) radical cations, respectively. The important observations are that DMF-\( d_1 \) undergoes a high-
energy rearrangement to give loss of HCO⁻, and three slow direct cleavages. The direct cleavages (with their average KERs [6]) are: loss of H⁻ (non-statistical, 0.19 eV); loss of CH₃⁻ (bimodal, 0.16 eV; and loss of DCO⁻ (statistical, 0.10 eV). We hypothesize that cleavage of the amide bond with loss of DCO⁻ occurs via a long-lived ion-dipole complex (Figure 2).

\[ C₂H₆N⁺ + DCO⁻ \]

Figure 2 (see text for details).

We have also begun to study other amide radical cations, and they also undergo some slow (metastable) direct cleavage reactions, none of which include cleavage of the amide bond. Both formamide and \( N \)-methylformamide lose H⁻; \( N,N \)-diethylformamide loses CH₃⁻. \( N,N \)-Dimethylacetamide undergoes no metastable decompositions, whereas \( N,N \)-diethylacetamide loses CH₃⁻; \( N,N \)-diisopropylacetamide loses C₂H₅⁻; and \( N \)-t-butylacetamide loses CH₃⁻.

We currently do not understand the chemistry of these interesting systems. Our on-going research includes obtaining evidence of the potential energy surfaces by modeling the KERDs by Phase-Space Theory and by evaluating the energies of the ionic structures by \textit{ab initio} calculations. We also plan to perform kinetic studies and model them by RRKM.

REFERENCES

6. We acknowledge Dr. P. A. van Koppen at UCSB for measuring the KERDs.
Single photon and Multiphoton Ionization of IR-Laserdesorbed Bio-Molecules


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Photoionization with lasers of different wavelength has resulted in a large number of applications in mass spectrometry. For ionizing molecules introduced into the gas phase two alternative methods can be applied. One possibility uses multiphoton [1] and the other single photon [2] absorption for ionization. While multiphoton ionization (MUPI) of laser desorbed biomolecules, like aminoacids or peptides, is a well tested technique, single photon ionization of IR-laser desorbed biomolecules is fairly new.

In this work we coupled the technique for generation of coherent 10.5 eV radiation (118 nm), which can be easily produced by frequency tripling the third harmonic of a Nd:YAG laser (355 nm) in a mixture of xenon and argon [3], to our Reflectron-Time-of-Flight (RETOF) mass spectrometer.

First mass spectrometric investigations show the application of single photon ionization to small CO2 laser desorbed peptides without chromophore groups. These molecules are not accessible by MUPI. All measured peptides give intensive ion signals with low noise. In every case the molecular ion can be observed but with intensities normally lower than 10%. Remarkable is the high intensity of the A1-fragment (Roepstorff-nomenclature [4]) which is often base peak.

The second measurements compare the two ionization techniques in combination with CO2 laser desorption of the aminoacid tryptophan and some tryptophan containing peptides. Overlapping in space and time of the VUV and UV foci and the separation of the third Nd:YAG harmonic were ensured. While the dipeptides Leu-Trp and Ala-Trp show nearly the same ionization pattern with single- and two photon absorption of the same energy, the mass spectra of tryptophan and the measured tri- and tetrapeptide are different. 10.5 eV single photon ionization of tryptophan gives the molecular ion and the methylindole fragment with a ratio of 1 : 2 whereas two photon ionization of the same energy produces only the methylindole fragment. We believe this may be due to a neutral dissociation after the absorption of one 236 nm photon.
More distinct differences exist in the spectra of Leu-Trp-Met and Leu-Trp-Met-Arg. In the first case single- and two photon ionization produce the molecular ion and a variety of different intense fragment ions. Multiphoton ionization of Leu-Trp-Met-Arg gives a nearly soft ionization. However, single photon ionization gives hard ionization with intense low mass fragment ions. We think this may be due to ionization of neutrals photoproduced during the desorption step as well as the large excess energy delivered to the molecular ion by the VUV-photon.

References


Acknowledgement

We gratefully acknowledge support of this work by the Bundesministerium für Forschung und Technologie (13N5307) and the Deutsche Forschungsgemeinschaft (GR917-1/2).
A Study of Peptide Fragmentation by Photon Induced Dissociation in a Magnetic Deflection Tandem Mass Spectrometer

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Department of Chemistry, MIT, Cambridge, MA, 02139 USA

We have previously demonstrated the feasibility of photon induced dissociation (PID) of relatively large peptides (500-2200) in a magnetic deflection high performance tandem mass spectrometer, operating in conjunction with a pulsed Cs ion gun, an excimer laser (400 Hz), and an integrating array detector. These promising but limited results prompted a more extensive study to evaluate the utility of lasers for both dissociation and ionization of peptides and proteins in a magnetic deflection mass spectrometer. We report here our most recent results using PID, at both 193 nm and 350 nm, as an alternative to CID. We also report our initial efforts at matrix assisted UV laser desorption.

EXPERIMENTAL

The PID experiments were carried out on a JEOL HX110/HX110 tandem mass spectrometer with a mass range of 14,500 at 10 kV accelerating potential. An excimer laser, operating at either 193 nm (ArF) or 350 nm (XeF), was used to photoexcite selected precursor ions in an excitation cell located in the interface between MS-1 and MS-2. The laser produces 12 ns pulses and was operated with a repetition rate of up to 400 Hz.

Photon Induced Dissociation To overcome the inherent difficulties associated with using a pulsed laser and a continuous beam ion source, the focusing lenses of a 25 kV Cs+ ion gun were interfaced with a square wave pulse generator. This pulsed source generates 5 µsec ion packets with a variable frequency. The precursor ions of interest are mass selected by MS-1 and transmitted into the photoexcitation interface. To improve the interaction cross section between the ions and the photons, the 10 kV ion packets are decelerated to 2 kV by a lens and the PID cell, which is floated at 8 kV. A pulse delay generator circuit linked to the Cs+ gun and the laser allows coordination of the arrival times of the ion packets and laser pulses. Because the velocity of the ion packets is inversely proportional to the square root of the mass of the ion, the timing is adjusted for each precursor ion mass. The laser beam is focused by a cylindrical lens resulting in a 25 x 0.5 mm profile which enters the PID cell through a quartz window, perpendicular to the ion beam. The energy of the laser can be varied reliably between 10 and 100 mJ per pulse.

After photon induced dissociation, the PID fragment ions are mass analysed by MS-2. Because the product ions are formed in a pulsed mode, the PID mass spectra are recorded using an integrating array detector. Integrations are varied between 0.1-10.0 sec.

The PID spectrum of peptides generated using a 193 nm laser source are in general, very similar to those generated with CID, at the same collision energy, using He as a collision gas. As was to be expected, the PID experiments performed using a 350 nm laser source were not effective until the peptides were derivatized with a chromophore which absorbs at 350 nm. The PID spectrum of the dinitrophenyl derivative (DNP) of PQGIAGQR shown in Figure 1 gave a complete set of C-terminal ions including abundant w7 ions. The most abundant ions were the w7 and the x7 which arise from cleavages adjacent to the DNP proline residue.

Figure 1
Matrix Assisted UV Laser Desorption. The laser desorption (LD) experiments were carried out using the FID cell as an ion source for MS-2. A small voltage was applied to the LD probe tip to act as a repeller. The instrument was operated at a resolution of 1000 and spectra were acquired using the array detector, either by jumping the magnet of MS-2 from m/z 200-4500 and connecting the segments to form a complete spectrum, or by setting the magnetic field so that the molecular ion of the protein falls at the array detector. Samples were dissolved in a saturated solution of dihydroxy benzoic acid (DHB), and 2-3 μl applied to the probe tip. Sinapinic acid was also evaluated as a matrix, but did not produce spectra beyond m/z 2000. The laser was operated at between 10 and 75 Hz at an energy of 20-200 mJ at the sample target, with the beam dimensions of approximately 25 mm x 0.5 mm at the plane of the target.

Figures 2a and 2b show the LD spectrum of the molecular ion region from 12 pmoles of Ubiquitin (M_r = 8564) and the molecular ion region for the doubly charged species from 4 pmoles of Myoglobin from horse skeletal muscle (M_r = 16,951) respectively. Both of these spectra show the presence of DHB photo adduct ions 136 u higher than the molecular ion. Figure 3 shows the full scan spectrum generated from approximately 32 pmoles of an unpurified chymotryptic digest of acetylated cytochrome C. This demonstrates that the ionization is not affected by either 2M urea or buffers.

Figure 2a

UBIQUITIN (PORCINE)
M_r = 8,564.8
12 pmoles

Figure 2b

MYOGLOBIN (HORSE)
M_r = 16,951.5
4 pmoles

Figure 3

CHYMOTRYPIC DIGEST OF ACETYL CYTOCHROME C M_r = 13,199
PROBING THE TRANSITION STATE OF n-PROPYL PHENYL ETHER ION DISSOCIATION BY TIME-RESOLVED PHOTODISSOCIATION

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Fairfield, CT 06430

and

Robert C. Dunbar, Department of Chemistry
Case Western Reserve University, Cleveland, OH 44106

The nature of the transition state for the decomposition reaction of alkyl phenyl ether ions to phenol molecular ion and the corresponding alkene has been the subject of a number of recent inquiries. Thermodynamic arguments and isotopic labeling results have been presented as support for the existence of an ion-neutral complex in the transition state rather than a cyclic McLafferty type intermediate.

The technique of time-resolved photodissociation spectroscopy is particularly useful in probing the kinetics of the transition state for decomposition with rate constants slower than $10^6$ sec$^{-1}$. The procedure involves comparing experimentally measured time-resolved data with theoretically generated RRKM fitted curves and from the RRKM plots extracting kinetic parameters such as the critical energy and entropy change at 1000 K.

Experiments were carried out on an ICR spectrometer with a one inch cubical cell, 1.4 T magnet and bridge detector. n-Propyl and i-propyl phenyl ether parent ions were produced by electron impact, allowed to cool for three seconds and then excited with laser light for 10 ns. Following a variable delay time during which decomposition proceeds the ICR detector sequence was initiated to yield the time-resolved spectrum. A Lumonics Nd:YAG laser was tuned to the 532 nm line to pump a dye laser producing 5 to 20 mJ 10 ns pulses at the desired wavelength of light between 727 and 650 nm.

Both isomers follow the generalized reaction below:

$$C_6H_5OR^+ \rightarrow C_6H_5OH^+ + R-H$$

RRKM fits for the n-propyl isomer yielded values of 1.24 eV for $E_0$ and -15 eu for.
$\Delta S_{1000}$ K. Results for the isopropyl isomer indicated that the decomposition was too fast on the ICR time scale for any kinetic parameters to be determined. Light intensity studies ruled out the possibility of any significant contribution from a two photon process. Figure 1 summarizes the thermodynamic data.

Previous work concluded that the n-propyl isomer rearranges to the isopropyl isomer as part of the decomposition mechanism. It is reasonable to expect that after this rearrangement the pathways for both isomers are identical. The striking difference between the decomposition rates for n-propyl and isopropyl isomers indicates that this rearrangement is the rate limiting step.

It is unclear whether the large negative entropy change for this reaction favors an ion-neutral complex for a concerted pathway. However, it is clear that the activated complex is tight.

References

KINETICS OF MULTIPHOTON PHOTODISSOCIATION OF IONS BY LOW-INTENSITY INFRARED LASER IRRADIATION

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Introduction

Ion dissociation by irradiation with a continuous, low-power infrared laser (cw IRMPD) is finding increasing use in ICR and QUISTOR ion traps. It is considered a very gentle dissociation technique, normally breaking only the weakest bond. We are trying to understand the kinetics of this process better through quantitative experiments, computer modelling, and theory.

We describe here the close analogy between cw IRMPD kinetics and thermal decompositions. The thermal analogy leads to Arrhenius activation energies for IRMPD; we discuss the relation between the activation energy and the dissociation energy of the ion. IRMPD data for n-butylbenzene ion and styrene ion are used to illustrate the application of these ideas.

Focus Question: A plot of dissociation rate versus laser intensity is intimately connected with the value of the dissociation energy (bond energy). How can we use these data to measure ion bond energies?

Computer Modelling

IRMPD was modelled by a computer simulation using a random walk on the energy axis. From these plots we calculate the predicted k_diss and induction time values as a function of laser intensity from 0 to 10 W.

The predicted results can be fitted to the experimental data to test the validity of the kinetic model assumed in the simulation, and to assign the temperatures for the thermal model.

Experimental Results

IRMPD of n-butylbenzene ions and styrene ions was studied in the FT-ICR instrument using a CO_2 laser at 940 cm^{-1}. A typical plot from the IonSpec data system is displayed, showing the decrease of parent ion and the increase of daughter ion, with dissociation commencing after an induction time. (Experimental data thanks to Guy Uechi and Rebecca Zaniewski.)
Thermal Picture of IRMPD

After the induction time, the laser-pumped ion population comes to a steady-state distribution. This distribution is closely approximated as a Boltzmann distribution truncated at the dissociation threshold $E_L$. The dissociation reaction occurs when an ion in the reactive zone ($E_f + \hbar \nu$ to $E_L$) absorbs one additional IR photon. This model leads to expressions for the activation energy, and to Tolman's theorem.

Activation Energies $E_a$: The activation energy defined by $E_a = R \frac{d \ln k_{diss}}{d (1/T)}$ is determined from the slope of the Arrhenius plot of $\ln k_{diss}$ vs. $1/T$, as shown in the figure. Values derived from the data are 0.32 eV (n-butylbenzene ion at 3.3 W), and 0.41 eV (styrene ion at 4 W).

The Arrhenius activation energies $E_a$ are much smaller than the dissociation thresholds $E_L$, which are 1.23 eV (n-butylbenzene ion) and 2.5 eV (styrene ion). We can understand this difference through Tolman's theorem (corrected for IRMPD) which says:

$$E_a = <E_{reacting}> - <E_{population}> + \text{small correction.}$$

Determining $E_L$ from plots of dissociation rate versus laser intensity: From rate versus intensity data alone, this is probably impossible. Using the induction time values to give additional information, as was done in the present study, this can be done through computer modelling. If independent ion thermometry information is available, an excellent determination of $E_L$ should be possible by a Tolman's theorem analysis of IRMPD data.

Conclusions

A satisfactory kinetic picture of cw IRMPD has been formulated, and its quantitative predictions calculated through computer modelling. The kinetics and the computer simulations are in excellent accord with experiment for n-butylbenzene ion and styrene ion.

The formulation of the kinetics using a thermal dissociation picture is successful, giving good agreement with the computer simulations and the n-butylbenzene ion results.

Determining threshold dissociation energies and ion bond strengths from IRMPD data is promising, but requires additional experimental information beyond the rate vs. intensity curve.
Introduction - A series of 9-Naphthyl Esters ranging from the Acetate (MW=186) to the Stearate (MW=410) have been studied using 10.5 eV photoionization and 266 nm multiphoton ionization. Ions were mass analyzed using a linear time-of-flight mass spectrometer and a microchannel plate detector. The basic fragmentation sequence for the esters is outlined in figure one. Primary and secondary fragmentation of the esters have been studied as a function of both molecular size and temperature.

Primary Fragmentation - Photoionization spectra for each of the esters were obtained using 10.5 eV radiation which was produced by frequency tripling 355 nm radiation in a Xe/Ar cell. The spectra show a parent ion and a primary fragment at M/Z 144. No secondary fragmentation was observed. A sample spectrum for 9-Naphthyl Myristate is shown in figure 2. The small amount of M/Z 115 present in the spectrum is the result of the absorption of residual 355 nm pump radiation in the source region. The 10.5 eV spectra were obtained at the same temperatures due to the rate dependence on temperature discussed below. Due to the varying volatilities two temperatures were used to obtain spectra for all of the esters. The spectra show a metastable tail on the high mass side of the M/Z 144 peak. Figure 3 shows the overlaid, normalized region around the metastable peak. By normalizing each of the spectra to the height of the promptly formed M/Z 144 ion, increases in the size of the metastable tail reflect a decrease in the ratio of prompt versus metastable ions (or $k_{\text{on}}$). The increase in the size of the metastable tail is roughly the same as the increase in the number of the vibrational degrees of freedom and thus only a weak function of size.

Figure 4 shows the temperature dependence of the metastable M/Z 144 tail for the 9-Naphthyl Laurate. The spectra shows increase in the prompt to metastable ion ratio with an increase in temperature and the magnitude of the increase is comparable to the molecular size increase.

Secondary Fragmentation - Multiphoton ionization spectra were obtained for each of the esters using 266 nm radiation. The absorption of two photons results in the production of a parent ion. Absorption of an additional photon results in both the formation of M/Z 144 and the metastable M/Z 116. The absorption of two additional photons by the parent ion results in the formation of M/Z 115. Figure 4 shows the expanded normalized region of the metastable tails for Caproate to Stearate. From this figure it is evident that there is no significant change in $k_{\text{on}}$ for the formation of M/Z 116 from M/Z 144 over this range of molecular sizes, and no "degrees of freedom effect" is observed. This result suggests that the parent ion internal energy may not be completely randomized before fragmentation.

Discussion - Statistical theories suggest that the parent ion decay rate should decrease quickly with increasing molecular size. This dependence is explained by simple RRK equations. Two extreme cases can be considered. In situation one, the parent internal energy (E) is held constant with increasing number of vibrational degrees of freedom (N). In this case k drops rapidly as N increases. However, no molecular size dependence would be observed if the fragmentation were driven by the thermal internal energy or if the energy is not randomized. This second situation would cause E/N to be constant and thus only a very small change in $k_{\text{on}}$ should be observed.
Figure One

Fragmentation Sequence for β-Naphthyl Esters

\[
\text{O} \quad \overset{0}{\text{C}} - \overset{n}{\text{(CH}_2)_n \text{C}_7H_7} \\
\]

\(n = 0 \text{ to } 16\)

\[ \rightarrow \text{C}_n\text{H}_m^+ \rightarrow \text{C}_n\text{H}_m^+ + \text{C}_j\text{H}_7^+ \]

Figure Two

10.5 eV Photoionization Spectrum
B-Naphthyl Myristate

Figure Three

Figure Four

Stearate (C₁₈)
Palmitate (C₁₆)
Myristate (C₁₄)
Lauroate (C₁₂)
ION THERMOMETRIC STUDY OF N-BUTYLBENZENE IONS: HEATING BY A LOW POWERED CW-CO\textsubscript{2} LASER AND SUBSEQUENT IR RADIATIVE COOLING

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Ion thermometry is the attractive idea of probing the average internal energy of an ion at any given time. Competitive photodissociation of n-butylbenzene ions has proven to be a very successful thermometric tool\(^1\). The branching ratio of photofragments m/z 91 and 92 as a function of ion internal energy is well established\(^1\). The ion internal energy can be probed simply by inducing dissociation by the absorption of a visible photon, and detecting the photofragments.

N-butylbenzene ions were produced in a Fourier transform ion cyclotron resonance spectrometer by electron impact. The ions were trapped for two seconds at 1x10^{-8} torr to assure thermal equilibrium had been reached with the temperature of the cell. After removing electron impact produced fragment ions the cw-CO\textsubscript{2} laser (3.3 W) irradiated the trapped ions with 940 cm\(^{-1}\) infrared (IR) photons. The heating of the ions by the IR light was probed by ejecting fragment ions produced by the IR irradiation, inducing photodissociation by a 10 ns visible laser pulse at 440 nm from a pulsed Nd-YAG laser, followed by excitation and detection of all ions. Having heated the ions to approximately 0.8 eV internal energy, the CO\textsubscript{2} laser was turned off. The cooling of these hot ions was observed by again probing the internal energy of the ions as a function of trapping time.
Figure 1 shows two processes due to irradiation by the CO₂ laser. The left axis displays the rise in parent ion internal energy as the ions sequentially absorb IR photons. The right axis shows the extent of dissociation of the ions due to IR multiphoton absorption. The dissociation curve shows the existence of an induction period during which the ions must gain enough energy before the onset of dissociation. The heating curve shows that the induction period corresponds to the time it takes the ion to reach its steady state energy. Another observation from the heating curve is the lack of an absorption bottleneck indicating that the thermal energy of the ions is sufficiently high to place the ions in the region of high density of vibrational states known as the quasicontinuum where incoherent absorption of IR photons occurs.

Figure 2 is the internal energy relaxation curve of n-butyl-benzene ions at 1 x 10⁻⁶ torr. Since collisions may contribute to the cooling rate at this pressure the radiative rate is simply determined by extrapolating to zero pressure in the pressure dependent study. With an initial excess internal energy of 0.45 eV the infrared radiative rate is 1.7 s⁻¹.

HYDROGEN TRANSFER AND STEREOCHEMISTRY IN ELECTRON IONIZATION MASS SPECTROMETRY OF TBDMS ETHER DERIVATIVES OF 3-HYDROXY STEROIDS.

John B. Westmore*, Helena Majgier-Baranowska*, Wayne D. Buchannon* and John F. Templeton
1

*Department of Chemistry, *Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

For 3-hydroxy steroids there are four stereoisomers, which differ according to the orientation of the hydroxy groups (i.e. axial or equatorial) and fusion (i.e. cis or trans) of rings A and B. All four isomers can be separated by gas chromatography of their tert-butyldimethylsilyl (TBDMS) ether derivatives on non-polar columns (Fig. 1). In EIIMS, the elimination of $\text{HMe}_2\text{SiOH}$ from the $\text{[M - t-Bu]}^+$ ion, reaction [1] (confirmed by observation of a metastable decomposition), depends strongly upon stereochemistry, occurring much more readily when the TBDMSO group occupies an axial position (Fig. 2A, 2C).

![Image of molecular structure]

To clarify the stereochemical requirements for this fragmentation, which involves a double hydrogen rearrangement and is potentially important in a predictive sense for other steroid geometries, we have synthesized selectively-labelled steroids with deuterium at specific positions (i.e. 1, 2, 3, 4, 5, 6, 7, 9a and 11) to establish the origins of the two transferred hydrogens. The results for one of these isomers are here discussed in more detail.

For 17a-methyl-17β-hydroxy-3a-O-TBDMS-5a-androstane, the origins of the two hydrogens eliminated in the neutral species of reaction [1] are: H(5α), 100%; H(6), 24%; H(7), 37%; H(9a), 46%. Although not confirmed by experimental evidence we presume that, for reasons given below, it is the α-hydrogens that are lost from positions 6 and 7. Thus, within experimental error, there is quantitative loss of H(5α), while H(6α), H(7α) and H(9α) together account for the second hydrogen lost.

Because the stereochemical information is not lost we assume that reaction [1] occurs without ring-opening. Molecular models confirm that H(5α) is ideally placed for transfer to Si through a six-membered cyclic transition state, to produce a tertiary carbo-cation at C(5). The formation of trigonal planar C(5) at the A/B ring junction reduces the pucker of both rings A and B. The molecular models indicate the closest distances that O(3) can approach to other hydrogens in the strain-free $\text{[M - t-Bu]}^+$ ion (structure I, scheme I) as indicated in Table 1, which also indicates the smaller distances obtained for sp$^2$ hybridization at C(5) (structures II and III).

![Table 1. Estimated distances of closest approach (Å).]

<table>
<thead>
<tr>
<th></th>
<th>C(5), sp$^2$</th>
<th>C(5), sp$^3$</th>
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</thead>
<tbody>
<tr>
<td>O(3) - H(9α)</td>
<td>2.45</td>
<td>0.9 (A boat, B chair)</td>
</tr>
<tr>
<td>O(3) - H(6α)</td>
<td>3.0</td>
<td>1.5 (A boat, B chair)</td>
</tr>
<tr>
<td>O(3) - H(7α)</td>
<td>3.15</td>
<td>1.7 (A boat, C chair)</td>
</tr>
</tbody>
</table>

Since the sum of the van der Waals radii of hydrogen (1.2 Å) and oxygen (1.4 Å) is 2.6 Å then a small "umbrella" vibration at C5 would be sufficient for O(3) to come within bonding distances of these hydrogens (and to no others), thus making hydrogen transfer possible. If we now invoke formation of an allyl cation as the driving force for reaction [1] what is necessary are appropriate 1,2-H and 1,2-methyl shifts, as shown in scheme 1.

ACKNOWLEDGEMENT. This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada, and the Faculty of Graduate Studies, University of Manitoba.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Figure 1. Gas chromatogram of a mixture of the four 17α-methyl-17β-hydroxy-3β-OTBDMS-5α-androstanes on a DB5 capillary column.

Figure 2. EI mass spectra of the four isomers.

Scheme 1
Angle-Resolved Neutralization-Reionization Mass Spectrometry

Aberra Fura, Frantisek Turecek*, Fred W. McLafferty
Chemistry department, Cornell University, Ithaca NY 14853-1301

The neutral product yields from collisionally activated dissociation (CAD) of neutrals in neutralization-reionization mass spectrometry (NRMS) are found to depend significantly on the z-axis scattering angle of the neutralization event. As shown by Cooks and co-workers [1] for CAD of ions, high scattering angles favor high-energy neutral products, which can be more characteristic of the isomeric structure.

In the Cornell tandem double-focusing mass spectrometer (EBEB) used, 10 keV precursor ions are mass analyzed by MS-I (EB), collisionally neutralized with Hg in the first collision cell, reionized in the second collision cell with O2, and mass analyzed by the second electrostatic analyzer to give NR spectra. The angle of the precursor ion beam in the xz plane is electrostatically determined just before collisional neutralization; those neutrals that have a z-axis velocity component consistent with the acceptance angle of MS-II exit slit are detected after reionization. A 70 V deflection reduces the NR signal intensities by approximately an order of magnitude.

With increasing scattering angle the NR spectra of 2-propenal show increased relative abundances of C2H2, C2H3, CO, C2H4, and CHO, generally favoring products of higher dissociation energies (Figure 1).

![Graph showing relative abundances in the NR spectra, Hg(70%)/O2(50%), of 2-propenal as a function of deflection potential.]

Figure 1. Relative abundances in the NR spectra, Hg(70%)/O2(50%), of 2-propenal as a function of deflection potential.

The angle dependent NRMS spectra of 1-butene, E-2-butene and 2-methylpropene also show (Figure 2) increased isomeric differences in the relative abundances of C4H6, C4H5, C3H5, and C2H4 at higher scattering angles, consistent with their dissociation energies to these species. The ARNR spectra of n-hexenes also show more differences at higher scattering angles reflecting

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the favored higher energy dissociations. It has already been shown that isomeric specificity of NR spectra can be greatly improved by using neutralization agents of low ionization potential [2] or CAD of neutrals [3]. These angle-resolved NR spectra offer a third, and inexpensive, alternative [4].

Figure 2. Relative abundances in the NR spectra, Hg(75%T)/O₂(50%T), of A: 1-butene, B: E-2-butene, and C: 2-methylpropene as a function of deflection potential.

References.
DISSOCIATION DYNAMICS OF m-IODOTOLUENE MOLECULAR ION: PHOTODISSOCIATION AND METASTABLE ION DECOMPOSITION

Joong Chul Choe*, Hun Young So**, and Myung Soo Kim
Department of Chemistry, Seoul National University, Seoul 151-742, Korea

Dissociation of m-iodotoluene molecular ion (reaction 1) has been investigated on a nanosecond time scale by a photodissociation (PD) technique.[1]

\[ m-\text{CH}_3\text{C}_6\text{H}_4\text{I}^+ \rightarrow \text{C}_7\text{H}_7^+ + \text{I}^- \]  (1)

Metastable ion decomposition (MID) has also been studied, as a means to investigate the reaction over an expanded time scale. The rate constant for reaction 1 has been determined by the photodissociation/mass-analyzed ion kinetic energy spectrometry (MIKES) method developed recently.[2]

The collision cell assembly of the ZAB-E mass spectrometer located in the second field free region was modified to produce an electric field of -5 – 5 kV/cm. The laser (Argon ion) beam was made to cross the ion beam at a position within the field. The translational energy of a photoproduced daughter ion after exiting the field region changed depending on the site of its formation. Hence, time-resolved dissociation of photoexcited parent ions could be investigated by analyzing the translational energy of daughter ions. A time resolution of around 1 ns could be achieved.[2] Charge exchange ionization (CS2/N2) was used to generate the molecular ion of m-iodotoluene with a rather well defined internal energy.

Both of the MIKE spectra for the reaction 1 occurring through PD and MID are composite, suggesting participation of more than one reaction pathway. Analysis of a MID/MIKE peak can be improved by acquiring the spectrum with an electrically-floated cell.[3] Kinetic energy release distributions (KERDs) for the PD and MID were determined using a rigorous numerical method based on ion-optical calculation.[4] KERDs derived for the PD and MID are shown in Fig. 1. The bimodal nature of these KERDs is apparent. The low and the high kinetic energy release (KER) components in the experimental KERDs may be associated with the reaction channels producing m-tolyl and tropylium ions, respectively. The surprisal analysis has been employed to separate the two components. KERD calculated for the m-tolyl ion formation using phase space theory is taken as a prior expectation. Experimental KERDs thus separated are shown together with the reconstructed KERD which is the sum of these. Integrating KERDs for each component, the ratio of the small KER component to the large KER one can be determined. From the comparison of experimental and theoretical KERDs for the two components it was found that the m-tolyl ion formation occurred statistically and the tropylium ion formation occurred with a considerable reverse barrier.

In the case of PD, the rate constant has been found to exhibit a broad distribution due to thermal internal energy distribution of the molecular ion. Most probable rate constant for PD at 488.0 nm has been found to be $(3.5 \pm 0.6) \times 10^7$ s\(^{-1}\). Rate constants for each channel of competing reactions could be obtained from the branching ratio determined through the analysis of the KERDs, which are plotted in Fig. 2 for the PD experiments at two wavelengths. Rate-energy curves for the two channels calculated by RRKM-QET are shown in Fig. 2.

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** Korea Standards Research Institute, Taejon 305-606, Korea.
Experimental rate constants on a microsecond time scale determined by MID are also shown in the same figure. Considerable negative values of activation of entropy in the RRKM-QET fitting were obtained for both of the competing reactions, suggesting that the rate constants are determined by the tight transition states. The m-tolyl ion formation can be understood by invoking the variational theory.

Figure 1. KERDs for reaction 1. (a) PD with 514.5 nm excitation, (b) MID. Points denote the experimental results. Curves are the separated and reconstructed KERDs from the surprisal analysis.

Figure 2. Rate constants for reaction 1. Filled and open circles are the experimental results for the formation of m-tolyl and tropylium ions, respectively. Solid curves a and b are the theoretical results obtained from RRKM-QET calculations for the formation of m-tolyl and tropylium ions, respectively.

References
INVESTIGATION OF Fragmentation MECHANISM OF 12-CROWN-4

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INTRODUCTION

In order to determine the mechanism of dissociation and structure of a fragment ion, one must know the chemical composition of that fragment. Chemical composition can be obtained using high resolution mass spectrometer. It is also important to know if the fragments are the result of a direct dissociation or if they are produced through consecutive reactions. Linked scanning, MIKES, and collision induced dissociation can provide information about consecutive reactions and therefore neutral counterparts. For those ions with similar chemical composition, one can also write several chemical structures, i.e., different isomeric forms. Photolization mass spectrometry provides information about appearance energies of the fragment ions from which the upper limit for the heats of formation can be calculated.

EXPERIMENTAL

VG/ZAB-E commercial mass spectrometer with OPUS data system has been utilized for the accurate mass measurement, linked scanning and metastable decomposition studies. Electron impact ionization with 70 eV kinetic energy have been used for these experiments. Reported chemical compositions have an accuracy of about one mDa or lower. Triple quadrupole mass spectrometer (Finnigan TSQ-70) was utilized for the CID study.

RESULTS

Chemical Composition. In the 70 eV electron impact mass spectrum of 12-crown-4 at low pressure the parent ion (m/z 176) intensity was never above the signal to noise level. Chemical compositions for several fragment ions of 12-crown-4 were identified using high resolution mass spectrometer. The results are listed in Table 1.

<table>
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<tr>
<th>m/z</th>
<th>Chemical Composition</th>
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<td>122</td>
<td>C_6H_{11}O_3^+</td>
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<td>53</td>
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</table>

Figure 1
**Direct and Consecutive Reactions.** At higher sample pressures ions with m/zs 176 and 177 were present, probably due to the self chemical ionization. To determine whether the fragment ions are products of a unimolecular dissociation or consecutive reactions, metastable decomposition of the parent ion and linked scanning of the parent ion and its major daughter ions (formed at higher pressure) were carried out. Results are shown in Figure 1, and Table 2. Collision induced dissociation of the major fragment ions were studied in a Finnigan TSQ 70 mass spectrometer using argon as a collision gas. Experimental parameters were optimized for the maximum efficiency, i.e., multiple collisions at energies of ca 10 eV. Results are tabulated in Table 3.

**DISCUSSION**

Results of the HRMS, linked scan at constant B/E and the CID indicate that at high energies, dissociation starts by the ionization of an oxygen atom of the 12-crown-4 molecule, and for most fragments this step is followed by an intramolecular hydrogen shift which opens the ring. Molecular ions then fragment via dissociation of different bonds along the molecular ion chain, as shown in Figure 3. On the other hand, as is shown in Figure 2, loss of C2H4O and C4H8O2 (or two consecutive loss of C2H4O) are the dominant processes at low energies. Two important features of metastable ion decomposition are that these ions possess a fairly narrow range of internal energies and this energy is very close to the threshold energy of the first dissociation channel. The result suggests that at low energies, i.e., 0.1-0.2 eV above the threshold for the dissociation of the 12-crown-4, simple bond cleavages are dominant, as shown in Figure 3. In general these results are in agreement with our previous photoionization study, i.e., they suggest a mechanistic dissociation of 12-crown-4. Formation of fragments by direct dissociation or consecutive reactions can be explained by simple bond cleavages, in which daughter ions do not necessarily have the most stable structures.

| Table 2. Linked Scan at Constant B/E for parent and Daughter ions of 12-Crown-4. |
|-----------------|-----------------|
| Selected Ion    | Major Daughters |
| 73              | 45              |
| 87              | 59,43,44,45     |
| 88              | 87,59,45,44     |
| 89              | 45              |
| 101             | 57,59           |
| 105             | 87              |
| 117             | 73,87,89        |
| 133             | 89,87           |
| 176             | 133,132,131,117,105,104,101,89,88,87,86,73 |

| Table 3. CID of the major fragments of 12-crown-4 |
|-----------------|-----------------|
| Selected Ion(m/z) | Products of the CID (m/z) |
| 122             | 89,73,45        |
| 117             | 89,87,45        |
| 101             | 57,45           |
| 89              | 61,45           |
| 88              | 58,57,45,44,43  |
| 87              | 59,43           |
| 73              | 45              |
INTRODUCTION

Electrospray (ES) mass spectra of recombinant bovine and porcine somatotropin (rBST and rPST) dimers were compared to their respective monomers with regard to ion intensity distribution, ion charge distribution and the effect of reduction of the dimer upon treatment with dithiothreitol (DTT). These comparisons were used as a means for probing the effect of molecular conformation on ion formation during electrospray ionization. ES/Mass Spectrometry (MS) was also evaluated for quantitating monomer in the presence of dimer.

EXPERIMENTAL

The rBST and rPST monomers were produced by biotechnology methods. The rBST dimer was isolated from a dimer rich waste stream obtained during isolation of rBST monomer. The rPST dimer was recovered from thermally stressed monomer solutions. The predicted MW's (in daltons) for these components are:

- Monomers: rBST 22116 (22120 reduced), rPST 22101 (22105 reduced);
- Dimers: rBST 44234, rPST 44204.

The ES/MS was performed on a SCIEX API III triple quadrupole mass spectrometer and circular dichroism on a Jasco Mark 5 spectropolarimeter.

ES/MS OF ISOLATED AND REDUCED rBST AND rPST DIMERS

The ES mass spectrum for the rBST dimer is illustrated in Figure 1. Three major ion distributions can be discerned in the spectrum, as indicated by the labels A-C, corresponding to MW's 44236±4 (rBST dimer, A), 44566±9 (rBST dimer + 330 daltons, B) and 22116±2 (rBST monomer impurity, C). Minor dimer components with MW's 43966±16 (A') and 44788±21 (B') were also observed. Note that the mass spacings between the dimer peaks are half that of the monomer peaks and that the monomer peaks completely overlap with the dimer peaks above m/z 1400 but below m/z 1400 the monomer peaks are isolated from the dimer peaks. Since the number of basic sites in a molecule determines the ion distribution in the ES mass spectrum, a linear relationship between molecular weight and the number of basic sites would predict the complete overlap of monomer peaks appearing in an ES mass spectrum with those of a dimer. The absence of complete overlap between rBST monomer peaks with those of the dimer peaks is probably due to conformational differences between the monomer and dimer such that the number of exposed protonatable basic sites are not proportional with molecular weight for the two structures. When the rBST dimer was reduced with DTT, the abundant ions observed in the ES spectrum (Figure 2) were consistent with MW's 22119±1 (rBST reduced monomer, D), 22367±2 (rBST reduced monomer + 247 daltons, E) and 21796±1 (rBST reduced monomer - 324 daltons, F). These data suggest that the rBST dimers principally were in reducible forms indicating that they were produced by disulfide exchange. A small amount of the unreacted starting dimers, G and H, were also observed. SDS-PAGE results, obtained under reducing conditions with beta-mercaptoethanol, indicated that 16% of the dimer did not undergo reduction.

Similar studies were performed with the rPST dimer. Electrospray results indicated that the dimer principally consisted of two components with MW's 44199±2 (rPST dimer) and 43889±5 (rPST dimer - 313 daltons) and a minor component with MW 43596±7. When the rPST dimer is spiked with rPST monomer, the lower mass monomer peaks can be clearly distinguished from the dimer peaks, as described above for rBST, suggesting again the effect of protein conformational differences on charge distribution of related monomers and dimers. Analysis by ES/MS of the DTT reduced rPST dimer indicated components with MW's 22106±3 (rPST reduced monomer) and 21788±2 (rPST reduced monomer - 317 daltons) were present suggesting that these dimers were also formed by disulfide exchange. A small amount of unreacted starting dimers were also observed. SDS-PAGE results, under reducing conditions with beta-mercaptoethanol, suggest that a significant portion of the dimer does not undergo reduction.

The secondary and tertiary structures of rPST monomer and dimer have been probed via circular dichroism (CD) spectroscopy. Changes in the secondary structure are determined by analysis of the intrinsic CD spectral region (180-250 nm) which for the rPST dimer and monomer were found to be identical. These results indicate that the alpha-helical content is the same for both proteins. The aromatic side-chain CD spectral region of rPST (250-325

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Similar studies were performed with the rPST dimer. Electrospray results indicated that the dimer principally consisted of two components with MW's 44199±2 (rPST dimer) and 43889±5 (rPST dimer - 313 daltons) and a minor component with MW 43596±7. When the rPST dimer is spiked with rPST monomer, the lower mass monomer peaks can be clearly distinguished from the dimer peaks, as described above for rBST, suggesting again the effect of protein conformational differences on charge distribution of related monomers and dimers. Analysis by ES/MS of the DTT reduced rPST dimer indicated components with MW's 22106±3 (rPST reduced monomer) and 21788±2 (rPST reduced monomer - 317 daltons) were present suggesting that these dimers were also formed by disulfide exchange. A small amount of unreacted starting dimers were also observed. SDS-PAGE results, under reducing conditions with beta-mercaptoethanol, suggest that a significant portion of the dimer does not undergo reduction.

The secondary and tertiary structures of rPST monomer and dimer have been probed via circular dichroism (CD) spectroscopy. Changes in the secondary structure are determined by analysis of the intrinsic CD spectral region (180-250 nm) which for the rPST dimer and monomer were found to be identical. These results indicate that the alpha-helical content is the same for both proteins. The aromatic side-chain CD spectral region of rPST (250-325
nm) is sensitive to changes in the tertiary structure. The CD spectrum shows a loss of intensity for the dimer as compared to the monomer which is indicative of a slight change in conformation between the two proteins. Therefore, the differences observed in the ion distributions of the ES mass spectra for the rPST dimer and monomer are consistent with the conformational differences determined by CD spectroscopy.

USE OF ES/MS TO QUANTITATE rBST OR rPST MONOMER IN PRESENCE OF DIMER

Since the lower mass rBST monomer peaks do not overlap with the rBST dimer peaks in the ES spectrum, the amount of monomer in the presence of dimer was estimated by use of calibration curves and by the standard addition method for rBST monomer peaks below m/z 1400. Figure 3 illustrates the calibration curves for rBST monomer for a variety of ions. Note the non-linearity of the calibration curves at high rBST monomer concentrations. In a 1 mg/ml sample of the rBST dimer containing some rBST monomer impurity, the observed ion[m/z] (intensity[counts]/scan) values were 1229.7 (4363), 1302.1 (10088) and 1383.2 (15941). Interpolating these intensity values on the corresponding calibration curve gives an average value of 0.047 mg/ml rBST monomer or 4.7% rBST monomer (w/w). The standard addition method gives a value of 3.7% rBST monomer (w/w). SDS-PAGE results based on the percentage of total stain for the 22000 dalton peak estimated 20% of the sample to be rBST monomer while analysis by gel filtration estimated 10% of the sample to be rBST monomer. In a similar fashion, the amount of rPST monomer present in the rPST dimer sample was found to be 2% (w/w) by ES/MS vs. 4% found by SDS-PAGE. These results demonstrate that quantitation for monomer present in dimer can be readily achieved by ES/MS because the low mass monomer peaks do not overlap with the respective dimer peaks as a consequence of the conformational differences that exist between rBST and rPST monomers and dimers.
ANALYSIS OF MONOCLONAL ANTIBODIES CONJUGATED WITH CHELATORS
BY MATRIX-ASSISTED UV-LASER DESORPTION MASS SPECTROMETRY AND
ELECTROSPRAY MASS SPECTROMETRY

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INTRODUCTION

A pharmaceutical use of monoclonal antibodies (MW ~150,000) actively being developed world-wide is for the targeting
of imaging reagents to the sites of tumors. The imaging reagents may be radioactive metal ions which bind to chelating agents
which are conjugated to the MoAb's through the ε-amino groups of the lysines. These small chelator molecules typically have
molecular weights between 300 and 1,500 daltons. The average number of moles of chelator conjugated to a monoclonal antibody
is referred to as the loading value of that conjugated chelator. Presently, a radioactivity trace assay or a titration assay is used to
quantitate the loading of the imaging reagents to monoclonal antibodies. A number of assumptions and experimental difficulties
are associated with both techniques that may result in inaccurate loading values. We have therefore evaluated matrix assisted UV-
LDMS and ESMS as methods for the determination of loading values. The LDMS loading values were computed from the
difference in the measured masses for the conjugated and untreated monoclonal antibody relative to the expected mass change upon
conjugation of one mole of chelator. Similar LDMS and ESMS experiments were performed after reducing untreated and
conjugated monoclonal antibodies with dithiothreitol to determine the mass of the light chains (MW ~24,000) and heavy chains
(MW ~50,000) of the monoclonal antibodies and the distribution of chelator between the light and heavy chains.

EXPERIMENTAL

The mass spectrometer used for analyzing the monoclonal antibodies was a reflector-type time-of-flight microprobe
instrument (LAMMA 1000) equipped with a Q-switched (7 ns) frequency-quadrupled Nd:YAG laser (Quantel YC 600S) which
operated at a wavelength of 266 nm with spot size diameters of 10-50 μm and irradiances between 10^6 and 10^7 W/cm^2. The
matrix used was sinapinic acid. The electrospray mass spectra were generated using a SCIEX API III triple quadrupole mass
spectrometer in solutions containing 10% acetic acid. All samples were desalted either by dialysis or gel filtration. The structures
of the conjugated MoAb chelators are illustrated in 1, 2 and 3.

RESULTS AND DISCUSSION

A) Matrix-Assisted UV-LDMS and ESMS Results for Pure and Reduced Monoclonal Antibodies

The measured masses computed from the LDMS and ESMS spectra for the pure and reduced monoclonal antibodies are
summarized in Table I together with the expected masses of the aglycosyl MoAb's obtained from the DNA sequences of the
respective components. The mass differences between the LDMS and DNA values for the respective components are attributed to
the average mass of carbohydrate present in the respective monoclonal antibodies. These data indicate that the carbohydrate is
principally present on the HC and absent from the LC, as expected. Note the excellent correlation for the light chains between the
predicted DNA masses and those measured by LDMS and ESMS. The HC's were very weak in the ES mass spectra. The peak
shapes for the molecular ions of both treated and untreated MoAb's were shown to be Lorentzian in the LDMS spectra.

B) Matrix-Assisted UV-LDMS and ESMS Results for Chelators Conjugated to Monoclonal Antibodies

Figure 1 illustrates a typical matrix-assisted UV-laser desorption mass spectra of monoclonal antibody chimeric B72.3
when pure (1A) and conjugated to a chelator (1B) and when reduced with DTT (Figures 2A and 2B, respectively). Table II
summarizes the measured masses for a number of chelators conjugated to chimeric B72.3 monoclonal antibody and compares the
LDMS loading values with loading values obtained by the radioactivity trace assay and titration assay. The LDMS loading values
tend to be higher than the respective loading values obtained by radioactivity trace assay and titration assay.

The conjugated chelators were treated with DTT to dissociate the chimeric B72.3 monoclonal antibody into reduced light
and heavy chains. The loading values for the individual light and heavy chains were computed (See the values in Table II). Since
chimeric B72.3 monoclonal antibody has 12 lysines on the light chain and 33 lysines on the heavy chain, the predicted
distribution for the chelator should be 27% on the light chains and 73% on the heavy chains.

Determination of loading values by ESMS was hampered by the fact that heterogeneous high mass MoAb samples
produce a continuum of peaks in the ES mass spectra and therefore the MoAb's must be reduced to LC's and HC's, smaller
measureable fragments. As indicated in Table II the individually measured conjugates for the LC's were obtained but no conjugates
for the HC's were observed (due to low sensitivity) which makes the calculations for the ES loading values for the whole MoAb's
unreliable.
Table I. Values of Monoclonal Antibodies and Their Reactivity with Heavy Chains

<table>
<thead>
<tr>
<th>Monoclonal Antibody</th>
<th>Experimental Value</th>
<th>Molecular Mass</th>
<th>Light Chain Mass</th>
<th>Reduced Mass</th>
<th>Reduced Heavy Chain Mass</th>
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<td>33,200</td>
<td>48,000</td>
<td>71,920</td>
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<td>Chimeric 271.3</td>
<td>LD DNA</td>
<td>146,000</td>
<td>33,200</td>
<td>48,000</td>
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</tr>
<tr>
<td></td>
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<td>1,100</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>147,000</td>
<td>33,200</td>
<td>48,000</td>
<td>71,920</td>
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<td></td>
<td>ES DNA</td>
<td>146,000</td>
<td>33,200</td>
<td>47,400</td>
<td>69,710</td>
</tr>
<tr>
<td></td>
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<td>1,100</td>
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</tr>
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<tr>
<td></td>
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<td>ES MAb</td>
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<td>1,100</td>
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</table>
Feasibility of GC High Mass Resolution Electron Capture MS for Quantitation of Toxaphene with Minimum Sample Preparation

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Introduction. Toxaphene is a mixture of ~670 chlorinated camphene derivatives. Saleh found 76% were polychlorobornanes and 22% polychlorobornenes. The complexity of Toxaphene prevents resolution of individual constituents by high resolution gas chromatography and the elution window for these compounds overlaps that of numerous other polychlorinated compounds prevalent in the environment.

Instrumentation and Conditions. All GC/MS runs were made using an HP 5890 gas chromatograph interfaced to a VG 70-250SE mass spectrometer. The combination ion source for electron impact and chemical ionization was used. Cold, on-column injections of 1 μl or 3 μl were made onto a 1 m, 0.53 mm id, retention gap, which was affixed to a 30 m, 0.25 mm id, 0.25 μm film, DB-5 fused silica column. The temperature program was that used by Swackhamer: 80°C for 1 min; 10°C/min to 200°C; 1.5°C/min to 230°C; 10°C/min to 280°C. The transfer line was maintained at 285°C; the ion source at ~130°C. The pressure of the Cl gas, CH3Cl, was adjusted to maximize the signal in the Electron Capture (EC) mode. The filament current was 200 μA; the accelerating potential was 8 kV.

Standards. Toxaphene and technical chlordane standards were obtained from the EPA Repository at the Environmental Monitoring Systems Laboratory, Las Vegas, NV. The internal standard, 1,000 was used. The quantitation ions, (M-Cl)−, were: m/z 311, 345, 377, 413 and 447.

Quantitation Ions. Three factors were considered in choosing the quantitation ions for Toxaphene: 1) full scan electron capture mass spectra were recorded to determine which ions were most abundant, 2) the most or second most abundant ion created from each polychlorobornene congener was used, and 3) of these two ions, the one that is subject to a lower interference from column bleed or residual calibration standards at a resolution of 1,000 was used. The quantitation ions, (M-Cl)−, were: m/z 311, 345, 377, 413 and 447.

The single selected ion recording descriptor contained, for each congener, a confirmation ion (M+Cl)− with a mass +2 or -2 amu different from the quantitation ion and a (M-Cl)- ion, which was used to check for contamination. If a peak in a quantitation ion trace were due to a major contaminant that contained a Cl atom, a much larger peak would appear in the (M-Cl)- trace and a correction could be made. No such corrections were necessary in this study.

The ratio of the sum of the areas under the ion chromatograms to the peak area for the internal standard was determined. A linear dynamic range was obtained for injections containing 50 to 50,000 pg of Toxaphene.

Interferences. The 3 most troublesome interferences cited by Swackhamer were: 1) technical Chlordane, 2) PCBs when oxygen is available in the source, and 3) polychlorodibenzophenyls (PCDPEs).

Swackhamer made area corrections for interfering chlordane ions. Most of the interference occurred for the m/z 342 quantitation ion. We used the m/z 311 ion instead. Over the elution windows shown above, chlordane was not a significant interference and no corrections were made for it.

Jansson and Wideqvist observed major interferences from PCBs by (M+Cl)- ions. While Swackhamer was able to eliminate the O2 necessary to create these ions in the ion source, we were not. We eliminated this interference by using a mass resolution of 10,000.

PCDPEs yield ions with the same molecular formula as the (M+Cl)- ions formed from PCBs. High mass resolution also separates the signal due to the (M-Cl)- ions of Toxaphene, e.g. C18(179)C18(127)Cl18 (376.0573), from the signal due to the (M+Cl)- ions from PCDPEs, e.g. C18(179)C18(127)Cl2 (376.0236). For this example, a resolution of 11,890 is required.

Interfering (M+Cl)- ions can be formed from tetra through octa PCB congeners. A 1:1:1 mixture of Aroclors 1242, 1254, and 1260 was added to a solution of Toxaphene to observe PCB interference. Many PCB isomers eluted within the Toxaphene quantitation windows.

Interference from PCBs as a function of mass resolution was studied over the elution windows determined from the Toxaphene standard. The ratio of the sum of the integrated Toxaphene signals to the internal standard signal (TOX/IS) was determined for triplicate GC/MS runs made with solutions containing several levels of the Aroclor mixture, 500 pg/μl of Toxaphene, and 10 pg/μl of the internal standard. Figure 1 is a histogram of the TOX/IS ratios. At resolutions of 1,000 and 5,000, greater interference occurred as the Aroclor concentration increased, while little interference was observed at resolutions of 10,000 and 15,000.

Removal of Unidentified Interferences. A local soil spiked with Toxaphene was extracted by supercritical fluid extraction (SFE) with CO2 and reconstituted into hexane. Displayed in Figure 2 are ion chromatograms for the method blank and soil extract acquired with mass resolutions of 1,000 and 10,000. Most of the interferences were eliminated at the higher resolution; much of the remaining signal might be due to a background level of Toxaphene in the soil.

Quantification of Toxaphene in a Soil. Toxaphene was added to an amber colored, methylene chloride extract of a soil that contained creosote. Deterioration in chromatographic resolution was observed, caused by other compounds in the soil extract. Only the three most abundant quantitation ions were used. A standard addition of 51 pg/μl of Toxaphene was made to this extract, which already contained 40 pg/μl. Triplicate GC/MS runs were made with a resolution of 1,000. The 51 pg/μl of added Toxaphene was underestimated by -8.2%. If a significant portion of the summed areas were due to interferences, the error would be positive. Because this was not the case, higher mass resolution would not improve quantitation of this particular soil extract.
Conclusions. High mass resolution (±10,000) can be used to remove interference with the quantitation of toxaphene by PCBs, polychlorodiphenylethers, and other compounds. Quantitation of toxaphene by standard addition without sample clean-up appears to be feasible. Further study with additional soil samples and extraction procedures is necessary to validate this conclusion. High mass resolution will be required when high levels of polychlorinated hydrocarbons that yield oxygenated ions in the source are present.

References

Although the research described in this abstract has been funded wholly or in part by the EPA Contract 68-CO-0049 to Lockheed Engineering and Sciences Co., it has not been subject to the Agency’s review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred.
DETERMINATION OF POLAR PESTICIDES AND THEIR BREAKDOWN PRODUCTS IN WATER SAMPLES USING THERMOSPRAY LC-MS

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Two liquid-liquid extraction (LLE) procedures with n-hexane-dichloromethane (1) and dichloromethane-ethyl acetate (2) and a liquid-solid extraction (LSE) procedure with C18 Empore extraction disks were used for the extraction of polar pesticides from water samples. The analytical protocol used for the extraction is cited below:

1-4L of water samples (surface water and artificial sea-water) containing polar pesticides with a resulting analyte concentration of ca. 25 ng/L were used. When the LLE procedure was used the pesticides were extracted first with 40 mL of n-hexane and after with 120 mL (60 mL x 2) of dichloromethane (for organophosphorus pesticides) and with 100 mL (33 mL x 3) of dichloromethane-ethyl acetate (50:50) + 0.2M ammonium formate (for the other compounds). In the case of using LSE the water sample was prefiltered to eliminate particulate matter and subsequently it was passed through the Empore extraction disk. 4 L of water were extracted with the vacuum adjusted to yield 2h extraction time which is a clear advantage over the cartridge that requires 4-8h (3).

RESULTS AND DISCUSSION

The recoveries and relative standard deviations (RSD) using LLE and LSE are listed in Table I. LSE gave much lower recoveries than LLE for the dealkylated atrazine metabolites which is explained by the highest polarity of these compounds and consequently least sorption capacity for the C18 disks with much lower breakthrough capacity. In addition, no differences in recoveries were noted when using either surface river water, drinking water and artificial sea-water samples.

In Fig. 1 the total ion current chromatograms obtained under TSP LC-MS with PI and NI modes of operation of the same water extract are shown. Under PI the chlorotropan pesticides and carbaryl exhibited higher sensitivity whereas for the organophosphorus pesticides the NI mode of operation was preferred. An exception was fenamiphos that exhibited better sensitivity in PI than in NI. Using PI mode the \([M + H]^+\) for chlorotropanes and the \([M + NH_4]^+\) for the other compounds ions are the base peaks in the TSP mass spectra. An exception is the organophosphorus pesticide fenamiphos that as a result of its amidate group exhibits a higher proton affinity than the ammonium ion and as a consequence \([M + H]^+\) is the base peak. The \([M + CH_3NH_2CO]^+\) ion obtained for carbaryl, has been previously reported (4) as a particular ion of N-methyl carbamates under TSP LC-MS conditions. The formation of other characteristic ions, such as \([M + CH_3CN + H]^+\) and \([M + CH_3COONH_4 + H - H_2O]^+\) follows a general formula proposed in a recent paper (4). Typical negative chemical ionization mechanisms were observed for organophosphorus pesticides (5). The specific fragments obtained were: the functional group + CH_3COOH, for tetrachlorvinphos, and \([M - R]^+\) (R being methyl or ethyl) and \([M]^+\) for parathion-ethyl and fenitrothion.

CONCLUSIONS

Although extraction efficiency of LLE can be higher than LSE with Empore disks for extracting certain polar pesticide metabolites, e.g., deethylatrazine and desisopropylatrazine, from water samples, the use of LSE is preferred since: avoids emulsifications, solvent consumption and potentially hazardous solvents are reduced. As an alternative to C18 cartridges, the Empore extraction disks have demonstrated the possibility of preconcentration up to 4L of water samples with shorter elution time. TSP LC-MS with PI and NI has been shown to be useful for the characterization of polar pesticides at levels of 12-30 ng/L in water extracts. When pesticides of different chemical groups are present, with different proton affinity values and/or with electron withdrawing groups that can stabilize the negative charge, the use of PI and/or NI is required.
TABLE I MEAN % RECOVERY AND RELATIVE STANDARD DEVIATION (RSD) OF PESTICIDES IN WATER USING LLE AND LSE WITH EMPORE EXTRACTION DISKS. Spiking level: 10-50 μg/L in 1-4 Liters of Water. n.d.: not determined.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>LLE</th>
<th>RSD</th>
<th>LSE</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrazine</td>
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<td>7</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>Cyanazine</td>
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<td>7</td>
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</tr>
<tr>
<td>Simazine</td>
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<td>7</td>
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<tr>
<td>Deethylatrazine</td>
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<td>25</td>
</tr>
<tr>
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<td>30</td>
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<td>Chlorpyrifos</td>
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<tr>
<td>Carbaryl</td>
<td>90</td>
<td>7</td>
<td>83</td>
<td>8</td>
</tr>
</tbody>
</table>

Figure 1. Reconstructed ion current chromatogram obtained in PI and NI modes TSP LC-MS of 1 L water extract containing 10-30 μg/L of (1) cyanazine, (2) simazine, (3) carbaryl, (4) atrazine, (5) fenithion, (6) fenamiphos, (7) tetrachlorvinphos and (8) parathion. LC eluent: methanol-acetonitrile-water (25.25:50) to methanol-acetonitrile (50:50) in 12 min at 1 mL/min. LC column: 4 μm LiChrospher 100 RP-18.

LITERATURE CITED
Continuous-flow membrane-dialysis thermospray tandem mass spectrometry for quantitation of drugs and metabolites in plasma

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Introduction

Tandem mass spectrometry (MS-MS) is a powerful tool in the rapid screening and quantitation of (groups of) target compounds in complex biological matrices. For the direct introduction of biological samples via an LC-MS interface, simple and versatile on-line sample pretreatment methods are needed. In this paper, the on-line combination of MS-MS and continuous-flow membrane dialysis is described. This combination allows the direct analysis of plasma samples containing a significant amount of macromolecular interferences. The potential of this method is demonstrated with the quantitative analysis of the aromatase inhibitor pyridoglutethimide (PG) in plasma samples of patients treated with PG. PG is under investigation as a potential analogue for aminoglutethimide, which is currently used in the treatment of postmenopausal women suffering from estrogen-dependent breast carcinoma.

Experimental

Plasma (1.26 ml) was introduced in an air-segmented donor stream (0.42 ml/min water) to the dialysis membrane (cut-off 10 kDa). The acceptor stream (0.60 ml/min water) was concentrated on a short trapping column (TC, 12 mm x 2 mm ID, packed with 40-63 μm C₁₈), which was subsequently desorbed. The eluate (1.2 ml/min 50% methanol in 50 mmol/l ammonium acetate) was transferred either directly to a Finnigan MAT TSQ-70 thermospray LC-MS-MS system or via an analytical LC column (100 mm x 4.6 mm ID, packed with 5 μm C₁₈) for thermospray LC-MS analysis. The thermospray system was operated in discharge-on mode (1 kV).

Results and discussion

Dialysis / concentration

In developing a system for continuous-flow dialysis various experimental parameters have to be optimized:

* selection of the packing material for the trapping column (based on the retention characteristics of the compound).
* concurrent or countercurrent dialysis.
* selection of the mobile phase to desorb the analyte from the trapping column and transfer it to the LC-MS interface.

The dialysis was performed in the countercurrent mode, while the trapping column was desorbed in the back-flush mode. The recovery for PG was ca. 33%.
Internal standard
For the quantitative analysis of PG in post-treatment plasma samples, glutethimide (Glu) was used as an internal standard. It was added to the samples prior to the dialysis step. Multiple reaction monitoring was used in the quantitative studies, using m/z=219 → 134 for PG and m/z=218 → 131 for Glu. No mutual interferences were observed in MS-MS.

Calibration plot for PG in plasma
Linear calibration was observed over two order-of-magnitude (3-300 ng/ml PG in plasma) using Glu as internal standard.

Pharmacokinetic curve
The levels of PG in plasma samples, taken from a patient suffering from breast cancer who was administered a single dose of 800 mg of PG orally, were monitored using continuous-flow dialysis in combination with MS-MS.

Discussion
The continuous-flow dialysis approach can be easily used for the monitoring of drug levels in biological samples without prior sample pretreatment. The method is used to remove macromolecular interferences from the sample in an online mode and provides selective sample pretreatment as well as preconcentration of the sample in the trapping column. Using the continuous-flow dialysis approach in combination with thermospray sample introduction and tandem mass spectrometry the quantitation limit for the parent drug is 5 ng/ml plasma. This is adequate for the monitoring of the typical levels found in clinical studies with PG. The analysis time is ca. 14 min per sample. After set-up the system can run automatically.

Conclusion
Continuous-flow dialysis in on-line combination with mass spectrometry is an easy, rapid and versatile method for the quantitative monitoring of drug levels in biological samples. The method is useful if the presence of macromolecular compounds in the biological sample prohibits the direct introduction of the sample to MS-MS via a thermospray interface. No further sample pretreatment is necessary. The determination limits that can be achieved, are compound-dependent (thermospray ionization); the method provides online sample preconcentration.

In favourable cases, the method can also be used for monitoring the levels of the metabolites by using coupling of dialysis and MS-MS. However, when isomeric metabolites are present in the samples, as in this case, the use of on-line LC-MS or LC-MS-MS is essential.
INTRODUCTION:
Aflatoxins are toxins that are members of a large group called mycotoxins. They are produced by the fungi Aspergillus flavus and Aspergillus parasiticus and have been found in various grains throughout the world where climatic conditions are favorable for their growth. The aflatoxins, especially aflatoxin B₁, are known carcinogens in many species of animals.

In the HPLC analysis of aflatoxins using fluorescence detection, aflatoxins B₁ and G₁ fluoresce naturally in aqueous solution while aflatoxins B₂ and G₂ do not. The reaction of aflatoxins B₁ and G₁, post-column with iodine at 70°C produces a compound of complex that fluoresces with an intensity similar to the fluorescence of aflatoxins B₂ and G₂. The objective of this study was to determine whether the reaction resulted in an iodine hydrated complex or a stable iodine derivative. It has been known for some time that fluorescence of aflatoxins B₁ and G₁ can be enhanced by reacting them with iodine, however, the reaction product has not been identified.

Kok et al. (1) determined aflatoxins (B₁, B₂, G₁, G₂) using post-column derivatization (PCD) with electrophoretically generated bromine and compared the results with that obtained with PCD using iodine. They proposed that the reaction between bromine or iodine with aflatoxin B₁ and G₁ was simply the breaking of the double bond on the furan moiety of the aflatoxin molecules and adding two bromine or iodine atoms.

Thermospray mass spectrometry (TSMS) was used in our study to determine (1) whether the reaction of iodine and aflatoxins B₁ and G₁ resulted in an iodine hydrated complex or a stable derivative, and (2) to identify the complex or derivative.

EXPERIMENTAL:
Thermospray mass spectrometry was performed using a Finnigan-MAT TSQ 70 triple quadrupole mass spectrometer equipped with a Finnigan-MAT thermospray probe and controller. Typically, the vaporizer was set to 110°C and the jet (aero) was set at 220°C. All analyses were performed in the positive ion, "filament off" and "discharge off" mode. Direct injection of samples was performed using an Altex model 100 dual head reciprocating pump and a Rheodyne 7125 injector equipped with a 100μl loop. The mobile phase was 0.1M ammonium acetate in water pumped at 1.20 ml/min. Typically, 20μl solutions of the samples were injected into the TSMS system for each analysis.

Thermospray tandem mass spectrometry (TSMS/MS) was conducted using the same conditions as for TSMS except that the collision gas was argon set to about 0.5 mtorr and the collision energy was set to -50 eV. The iodine aflatoxin products were injected individually and the daughter ion spectra were obtained by setting ms1 to pass the appropriate [M+H]⁺ ion and operating ms2 in the full scan mode.

Derivatization of aflatoxins B₁ and G₁ was performed by reaction of the selected aflatoxin with saturated aqueous iodine in methanol/water (50/50) at approximately 70°C for 3 minutes.
RESULTS and DISCUSSION:
The TS mass spectra of the iodine derivatives of aflatoxins B$_1$ and G$_1$ gave surprising results. The [M+H]$^+$ ion for the iodine derivative of aflatoxin B$_1$ was at m/z 471. The [M+H]$^+$ ion for the iodine derivative of aflatoxin G$_1$ was at m/z 487. If two iodine atoms had added across the double bond on the furan ring as suggested by Kok et al. (1), the molecular weight for the aflatoxin B$_1$ derivative would have been 566. Also, if only one iodine and one hydroxy group from the mobile phase had added across the double bond of the furan ring, the molecular weight for the aflatoxin B$_1$ derivative would have been 456.

The observed [M+H]$^+$ ion at m/z 471 for the aflatoxin B$_1$ derivative can be explained by the addition of one iodine atom and a methoxy group across the double bond of the furan ring of the aflatoxin molecule (See Figure 1). More evidence is given for the proposed iodine and methoxy group attachment to aflatoxin B$_1$ by the presence of a peak at m/z 344 which can be attributed to the [M+H - I]$^+$ ion.

TSMS analysis of the aflatoxin B$_1$ derivative formed in the presence of deuterated solvents (CD$_3$OD and D$_2$O) gave more evidence of the methoxy group attachment because the [M+H]$^+$ ion observed for the aflatoxin B$_1$ deuterated derivative was at m/z 474. This increase of 3 daltons corresponds to the attachment of a CD$_3$O group as compared to the attachment of a CH$_3$O group.

TSMS/MS analysis of the [M+H]$^+$ ion at m/z 471 for the aflatoxin B$_1$ derivative provided daughter ions corresponding to losses of 128(m/z 343), 156(m/z 315), 159(m/z 312), and 188(m/z 263), which can be attributed to the [M+H - HI - CO]$^+$ ion, the [M+H - HI - CH$_3$O]$^+$ ion, and the [M+H - HI - CH$_2$H$_2$O$_2$]$^+$ ion, respectively.

The TSMS and TSMS/MS results provide strong evidence that a stable iodo-methoxy-derivative is formed when aflatoxins B$_1$ and G$_1$ are reacted with iodine.

![Figure 1](Image)

Proposed reaction product obtained with aflatoxin B$_1$ and iodine.

REFERENCES
ENERGY CONVERSION IN COLLISION-INDUCED DISSOCIATION OF POLYATOMIC IONS AT
LOW AND INTERMEDIATE COLLISION ENERGY.
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An unique tandem hybrid mass spectrometer utilizing a supersonic jet molecular beam source of neutral collision
gases is providing interesting new insights into the role of internal and collision energy on the mechanisms of activation
and dissociation of model systems. The observation of superelastic scattering of aceton ion led to the discovery of a
long-lived electronically-excited state of the aceton molecular ion whose dynamics have now been completely
characterized. This system provides an interesting example of microscopic reversibility in reaction dynamics as well as
providing the key insight that translational and internal energy are strongly coupled in low energy collisions under
appropriate circumstances. Further studies in our laboratory and elsewhere on CID of molecular systems have
demonstrated that electronic excitation plays a role in CID excitation for hydrogen, methane, acetone, nitromethane and
benzene---e.g., in most of the studies to date on CID reaction dynamics. Hence it is already clear that efficient T<\rightarrow E
conversion can be an important general mechanism in collisional activation of ions at low energy.

The benzene cation is another example—like acetone and nitromethane—for which the photoelectron spectrum
is quite "coarse grained" with gaps between overlapping electronic bands in which it is quite difficult to excite and ionize
the molecule. In all three cases our CID dynamcis study demonstrates a strong dependance of CID excitation mechanism
configuration. This contrasts sharply with propane, for which overlapped bands with no gaps characterize its photoelectron
structure as a preliminary step in NO formation. We have also demonstrated the microscopic reversibility concept in reaction kinetics/dynamics by translationally pumping ground state ions into the excited state manifold as well as translationally inducing the de-excitation of this electronic state. Further analysis of details of the observed scattering contour diagrams shows that dissociation occurs from both electronically-excited and ground state potential surfaces for acetone and the relative probability of these competing processes is a strong function of collision energy.

The propane ion has now been investigated in almost as much detail. In strong contrast to acetone, it exhibits
small and regular changes in its CID dynamics as collision energy is varied. At low and intermediate energy the most
probable scattering angle and energy deposition functions both correlate linearly with the general reduced scattering angle
parameter, \( \varepsilon \). This linear behavior is no longer observed as energy increases without limit, and a "saturation" behavior is eventually observed in scattering angle. This saturation occurs at the limit where toward scattering with impulsive excitation are the characteristic excitation mechanism. For this archetypical RRKM\textit{\rightarrow}QET molecular ion we also conclude that the Massey adiabatic criterion hypothesis adequately rationalizes the energy depositions in CID at all energies. Convolution of this function with the experimentally-determined breakdown graph predicts both the product ion distribution as a function of collision energy and the average energy deposited in the molecular ion for each individual ion product.

Further analysis of details of the observed scattering contour diagrams shows that dissociation occurs from both
electronically-excited and ground state vibrational surfaces for acetone and the relative probability of these competing processes is a strong function of collision energy.

The nitromethane cation is an intriguing example of a system whose mass spectrum involves several
rearrangements into highly stable cation intermediates separated by energy barriers with several transition states which are
loosely described as ion--dipole complexes. These adiabatic pathways are not traced in the CID of this ion; rather it
appears that a diabatic pathway is followed by highly-excited ground state nitromethane ions which surmount the lowest
energy barrier, leading to NO formation. Three dynamically distinct mechanisms are followed, only one of which is in
competition with \( \text{NO}_2 \) formation on the ground state hypersurface. At and above 18 eV dissociation to NO on an
electronically-excited hypersurface (level 6) becomes the dominant mechanism. These features are all consistent with the
photoelectron spectroscopy and experimental breakdown graphs for nitromethane and methyl nitrite cations, coupled with the assumption that crossing a barrier leads to rearrangement of highly excited nitromethane ions to the methyl nitrite structure as a preliminary step in NO formation.

The benzene cation is another example---like acetone and nitromethane—-for which the photoelectron spectrum
is quite "coarse grained" with gaps between overlapping electronic bands in which it is quite difficult to excite and ionize
the molecule. In all three cases our CID dynamics study demonstrates a strong dependance of CID excitation mechanism
on collision energy, with the possibility to "switch--on" and "switch--off" a mechanism by tuning the kinetic energy of the
colliding species. These are characteristic features of "curve--crossing" mechanisms in which the probability of a transition from
one hypersurface to another is strongly dependant on curvature of the surface and the rate of passage through that
configuration. This contrasts sharply with propane, for which overlapped bands with no gaps characterize its photoelectron
structure as a preliminary step in NO formation.
spectrum. We suggest that this is the primary reason that the CID dynamics for propane are relatively featureless and quite different from the other molecules we have studied.

Our attempt to generalize these observations leads to the suggestion that CID dynamics of polyatomic ions may be generally classified in three categories, depending on the "collision time" as a primary parameter. At the low energy limit adiabatic behavior is expected and detailed features of potential hypersurfaces will be important. Here the collision partner will play a very important role and "reactive gases" will be interesting activators. In an intermediate energy regime adiabatic surface crossings will be important and the dynamics may change rather dramatically with collision energy and scattering angle and will depend strongly on the individual properties of the ion investigated. At high energy the scattering gas is of little importance (in the center-of-mass frame, the mass effect on dynamics is critically important at high laboratory energies in terms of both energy deposition and scattering angle) in that small impact parameter impulsive collisions become the dominant CID excitation mechanism. In this limit the rotational times and vibrational times are both longer than the collision time and an averaged ensemble of particles undergo essentially "billiard-ball" collisions with strong forward scattering of the detected particles.

Support of this research by the National Science Foundation, Grant No. CHE-8312069, is gratefully acknowledged.
Fragment ions decrease steadily in abundance as larger and larger molecules are ionized because, as an ion gets larger, the energy put into it gets dispersed over more degrees of freedom and is less frequently in the reaction coordinate. According to RRKM calculations (by LLG), the average lifetime of a protonated oligomer of 191 alanine-glycine units (MW = 111.7 kD) containing 2930 kJ mol⁻¹ (approximately the thermal energy at 298 K in the neutral molecule plus that added by absorption of a 193 nm photon) would be 30 years if its lowest decomposition threshold were 105 kJ mol⁻¹. Adding more energy does not seem to be a feasible way to overcome the degrees of freedom effect. Therefore mass spectroscopists should turn to chemical reactions with ionized macromolecules to obtain characteristic fragments; i.e., gas phase analogs of the Edman degradation of peptides are needed.

Endothermic ion-molecule reactions can provide sequence information from protonated peptides [1]. This is a promising start in developing an ion-molecule chemistry for structural determination of biological macromolecules. Such reactions will take place through complexes between the ions and reagent neutrals. Thus understanding complex-mediated reactions is a prerequisite to using ion-molecule reactions to characterize macromolecules.

Extensive studies over the past decade on unimolecular complex-mediated reactions reveal much about their characteristics [2]. The following equation [3] indicates the factors that determine complex stability, and hence the importance of reactions mediated by a particular complex.

\[ \omega(\mathbf{r}) = -\mu q \cos(\theta)/r^2 - q^2/2\alpha + L^2/2ur^2 \]

\( \mu \) is the dipole moment of the neutral, \( q \) is the charge on the ion, \( \theta \) is the average angle between the dipole moment and the line of centers of the collision, \( r \) is the distance between the partners, \( \alpha \) is the polarizability of the neutral, \( L \) is the orbital angular momentum of the partners, and \( \mu \) is the reduced mass of the partners. According to this expression, the stability of the complex will increase with increases in the dipole moment and polarizability, and decrease with the effective distance between the center of charge and the neutral.

The polarizability of neutrals increases with size, suggesting that complex-mediated reactions will become more important with increasing size of the neutral, all else being equal. This is supported by the effect of increasing the size of alkyl radicals on complex-mediated alkane eliminations from ionized ethers [4].

The metastable decompositions for R'H elimination from a series of ionized ethyl 2-alkyl ethers increase in importance as R' is made larger. Photoionization ionization efficiency (PIE) curves demonstrate a parallel increase in the energy range of R'H elimination [4]. Thus complex-mediated reaction increase in importance with increasing polarizability of the neutral partner.

Increasing the size of the ion in the complex should increase the average distance between the center of charge and the neutral. Consistent with this, increasing the size of R in the ionized ethers decreases the abundance of metastable alkane eliminations [4]. PIE curves demonstrate that R'H elimination diminishes relative to loss of R as R is lengthened.
Changing the location of the complex-forming bond cleavage in isomeric ions will change r and therefore the importance of complex-mediated fragmentation. To explore this, we determined that ratio $\frac{r}{c}$ in the metastable decompositions of a series of ethyl branched $C_{n}H_{2n}$ isomers. The ratio was 0.31 when the ethyl was cleaved from the 2-position and 5.3 when the ethyl was cleaved from the middle (5) position. This difference could be due to a substantial difference between the center of mass and center of charge in the complex when ethyl is cleaved from near the end, and the approximate coincidence of these centers when bond cleavage occurs near the middle of the system. In the first case more then in the second, rotation of the partners will tend to move the ethyl away from the hydrogen it abstracts upon ethane elimination.

The dipole moment of the neutral should strongly influence complex-mediated dissociations (Eq 1). To test this, we characterized the dissociations of ionized 2-methylpropanol.

\[
\begin{align*}
\text{CH}_3\text{CHCH}_2\text{OH}^+ & \rightarrow \text{CH}_3\text{CHCH}_3^+ + \text{CH}_2\text{OH}^+ \\
\left[\text{CH}_3\text{CHCH}_2\text{OH}^+\right] & \rightarrow \text{CH}_3\text{CHCH}_2^+ + \text{CH}_3\text{OH} \\
\left[\text{CH}_2\text{HC} = \text{CH}_2^+\right] & \rightarrow \text{CH}_2\text{CHCH}_2^+ + \text{CH}_3\text{OH}^+
\end{align*}
\]

The complex-mediated products $\text{CH}_3\text{CHCH}_3^+$ and $\text{CH}_3\text{CHOH}^+$ are about 50% as abundant at 70 eV electron energy as the most abundant ion $C_{n}H_{2n}$. PIE curves reveal that these complex-mediated dissociations are strongly competitive with simple dissociations up to at least 190 kJ mol$^{-1}$ above threshold. In marked contrast, eliminations through ion-nonpolar neutral complexes are important only very close to the threshold for simple dissociation [4]. Thus a substantial dipole moment in the neutral partner appears to strongly enhance complex-mediated reactions.

Our work suggests that polar neutrals should be selected to get reactions between small molecules and large ions. Complex-mediated reactions tend to be confined to narrow energy ranges, so relative translational energies need to be selected with this in mind. Another problem may be that proton transfer reactions can sometimes occur to the exclusion of bond-forming reactions in ion-molecule reactions [5].

ENDOTHERMIC ION-MOLECULE REACTIONS:
FROM PROTON AFFINITIES TO PEPTIDE SEQUENCING

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Charge-exchange reactions were among the earliest applications of the tandem mass spectrometer constructed by Lindholm in 1954 (1). In this instrument, low primary beam energies were produced by a focusing, deceleration lens system, the incoming beam, in order to select for those ions not carrying appreciable forward momentum. A similar configuration was constructed some years later by Weiner, et al. (2), and was used by one of us (RJC) for the study of endothermic ion molecule reactions: D₃O⁺ (D₂⁺ OD) D⁺ and D₃O⁺ (D₂⁺ D₂O) D⁺ involving proton transfers via a long-lived collision complex (3,4).

Phase space theory, introduced by J.C. Light (5) in 1965, suggested that product ion yields decreased at higher beam energies as additional dissociation channels of the collision complex are opened up. This led to our consideration of the possibility that fragment ions from peptides might be formed as alternate dissociation channels from a collision complex formed during an endothermic proton transfer reaction between the protonated peptide and a less basic target gas. In our earliest studies (6), protonated leucine enkephalin (MW 555) was reacted with ammonia. Proton transfer and formation of the collision complex were observed at beam energies above 6 eV (Figure 1a), while sequence ions were observed at slightly higher energies (Figure 1b). We observed a similar threshold for a cyclic, hydrophobic peptide, suggesting that the endothermicity could be estimated assuming transfer of a proton located on the amide bond to ammonia (7). Conversely, peptides containing basic residues (histidine, lysine or arginine) did not react with ammonia to produce either proton transfer or dissociation
prior to the onset of direct collision induced dissociation (CID), since such reactions would be more endothermic (7). The assumption that the endothermicity corresponds to transfer of a proton from the amide bond was further tested by subsequent reaction induced dissociation (RID) studies of N-acetylated glucosamine oligosaccharides (8).

In our current studies, we have carried out RID reactions between peptides containing the basic residues histidine and lysine with dimethylamine. In Figure 2a, low beam energy collisions between angiotensin (which contains the histidine residue) and ammonia results only in the slowly rising fragmentation expected from direct CID processes. In Figure 2b, the reaction between angiotensin and dimethylamine produces additional, abundant fragmentation due to reaction induced dissociation, whose onset corresponds closely to the predicted threshold for the proton transfer reaction. Similar results were obtained from the reaction between Leu-Trp-Met-Arg-Phe-Ala and trimethylamine.

Reaction induced dissociation, utilizing endothermic ion-molecule reactions, provides an alternative method for inducing fragmentation at very low beam energies. In future studies, we expect to extend this method to larger peptides and other biomolecules.

CID OF CLUSTER IONS: WHICH RESULT SHOULD ONE BELIEVE?

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In 1980 Maquestiau et al.[1] reported that the adduct ion formed between the ammonium ion and ketones under ammonia CI conditions fragmented, after high energy collisional activation, by loss of neutrals of 17 (NH\textsubscript{3}) and 18 (\?) amu. They suggested that the neutral of 18 amu was water. Since the proton affinity of ammonia is greater than that of ketones a simple proton-bound cluster ion 1 would be expected to give NH\textsubscript{4}\textsuperscript{*} and they concluded that the ammonium ion had added to the carbonyl to give the carbinolamine 2.

\[ R_1\text{C}=O--H'--NH_2 \quad \text{and} \quad R_2\text{C}=O--NH_3 \]

They further concluded that the higher cluster containing two ammonia molecules was a protonated carbinolamine solvated by ammonia.

Figure 1 records high energy CID mass spectra for the (CH\textsubscript{3})\textsubscript{2}CO.NH\textsubscript{4}\textsuperscript{*}, (CD\textsubscript{3})\textsubscript{2}CO.NH\textsubscript{4}\textsuperscript{*} and (CH\textsubscript{3})\textsubscript{2}CO.ND\textsubscript{4}\textsuperscript{*} adducts. The major fragmentation of the unlabelled adduct ion is by loss of neutrals of 17 and 18 amu as reported by Maquestiau. The middle spectrum shows that all hydrogens lost with these neutrals come from the ammonium ion. The formation of m/z 58 in the bottom spectrum indicates considerable loss of ND\textsubscript{4} (or ND\textsubscript{3} + D) and shows that much of the loss of 18 amu in the unlabelled case must correspond to loss of NH\textsubscript{4} or NH\textsubscript{3} + H, rather than water as suggested earlier. MS/MS/MS experiments indicate that the m/z 58 in the unlabelled case is the acetone molecular ion.

Figure 2 compares the 8 keV and 50 eV CID mass spectra of (C\textsubscript{2}H\textsubscript{5})\textsubscript{2}CO.NH\textsubscript{4}\textsuperscript{*} adduct. In the high energy CID mass spectrum there is little formation of NH\textsubscript{4}\textsuperscript{*}, rather fragmentation occurs by loss of NH\textsubscript{3} and loss of NH\textsubscript{4} (NH\textsubscript{3} + H). By contrast, in the low energy CID mass spectrum NH\textsubscript{4}\textsuperscript{*} is the only significant product observed. Figure 3 shows the 8 keV CID mass spectra of the adducts of diethyl ketone with protonated methyl, dimethyl and trimethyl amines. Note that no loss of H\textsubscript{2}O is observed but rather loss of amine and amine plus H. In all three cases the low energy (50 eV) CID mass spectra showed only formation of the protonated amine. With increasing methyl substitution of the amine the loss of amine decreases in importance in the high energy CID spectra and formation of the protonated amine increases in importance, with the result that the low and high energy CID mass spectra are in agreement for the adduct with trimethyl amine.

The question arises as to how these differences between the high and low energy CID spectra originate and which spectrum one should believe when assessing structures. We believe that the results presented above as well as results for higher clusters can best be rationalized by assuming in all cases that a simple proton-bound cluster ion is formed. On low energy collisional activation, where vibrational excitation occurs, fragmentation proceeds as...
expected with the proton being found with the species of higher proton affinity. We propose that in the high energy CID electronic excitation of the ketone occurs and that fragmentation occurs before this electronic excitation is dissipated. It is known from solution studies that the electronically excited ketone has a greater proton affinity than the ground state ketone [2]. Thus it is able to compete more effectively with the amine for the proton, but loses out in this competition as we increase the methyl substitution of the amine and thus its proton affinity.

A full account of this work has been submitted to J. Am. Chem. Soc.

As part of our recent efforts to develop and apply quadrupole ion trap techniques to multiply charged ions derived from electrospray (1-3) we have embarked on a study of the ion/molecule reaction chemistry of multiply charged ions (4,5). To date, these studies have been restricted primarily to gas-phase Bronsted acid-base reactions and, within this class, to reactions of multiply protonated molecules with small gaseous bases. We have, however, studied a limited number of reactions involving proton transfer from gaseous acids to multiply charged anions. The goals of this effort are to understand fundamental aspects underlying the phenomenology of these reactions and to identify, maximize, and exploit the information that they can provide.

All experiments were carried out with a Finnigan-MAT ion trap mass spectrometer modified to allow ions formed by electrospray to be injected into the ion trap (1). The procedures necessary to form, inject, and analyze multiply charged ions have been described (1-3). For ion/molecule reaction studies a gas-phase base is admitted into the vacuum system to a pressure of roughly 1 x 10\(^{-7}\) torr. At this pressure, a mass spectrum can be obtained with minimal effect of the base provided the time between ion injection and mass analysis does not exceed roughly 50 ms. However, with delay times on the order of a few hundred ms reactions with rate constants on the order of 10\(^{-9}\) cm\(^3\)-molecule\(^{-1}\) cm\(^{-1}\) can proceed largely to completion. Gas-phase bases used to date include in order of increasing basicity: ammonia, dimethylamine, 1,6-hexanediamine, and N,N,N',N'-tetramethyl-1,8-naphthalenediamine.

All of the results observed thus far can be rationalized as involving the formation of a proton-bound complex. The fate of the complex determines whether or proton transfer takes place, adduct formation occurs, or no net reaction is observed. Reactivity can be understood qualitatively with reference to the energy diagrams of Figure 1 which show the combined energy of the reactants approaching from the left (entrance channel) and the products exiting to the right. The potential well in the center corresponds to the proton-bound dimer ion. Note that the reactants approach over an attractive surface (ion-induced dipole attraction) whereas the products experience a repulsive field due to charge separation. The exit channel therefore has a significant reverse critical energy, unlike proton transfer reactions of singly charged ions. Since there is a significant reverse critical energy in one of the channels, the occurrence or non-occurrence of proton transfer is not determined by thermodynamics but by the relative critical energies for the break-up of the cluster. In the top example of Figure 1 rapid proton transfer is expected because the reactants enter with energy well in excess of the critical energy for proton transfer. In the bottom example no reaction is expected because the reactants cannot, without some kinetic excitation, overcome the barrier to proton transfer. The intermediate case, where critical energies for cluster break-up are nearly equal, there are many degrees of freedom, and there is a deep well, is most conducive for clustering. The lifetime of the complex is maximized under these conditions thereby maximizing the likelihood for collisional stabilization of the cluster. An example of a clustering reaction is given in Figure 2 which shows the electrospray mass spectrum of bovine insulin in the absence of a gas-phase base (Figure 2a) and after the ions are allowed to react with 1,6-hexanediamine (Figure 2b).

In many ways these reactions are similar to reactions of small singly charged ions. However, major differences include the unusual shape of the energy hypersurface due to the coulombic field and the unusually high number of degrees of freedom of the ions. The shape of the energy hypersurface over which the reactions proceed is determined by the strength of the base, the intrinsic strengths of the protonated sites of the ion, the degree to which protons are multiply bound within the ion, and the relative locations of the charges. The latter factor, which determines the contribution of coulombic repulsion to the energy hypersurface, is expected to be particularly important as the charge state increases and this is consistent with observation.
In addition to their intrinsic appeal, these reactions have several useful applications. For example, we have demonstrated that charge can be concentrated into a few charge states via proton transfer reactions to increase signal/noise, we have demonstrated the use of proton transfer and clustering reactions for product ion charge state determination following collision-induced dissociation (5), and, in some cases, we have been able to determine the most likely sites of protonation in multiply charged peptides and proteins from the relative reactivities of the various charge states and those of product ions. It seems likely that as our quantitative understanding of these reactions grows, further practical applications may become available.


REFERENCES


Bovine Insulin

no base added

1,6-hexanediamine

(2x10^-7 torr, 0.5 s)

Figure 1

Figure 2
A COMPARISON OF NANOSCALE CAPILLARY LC AND CZE IN COMBINATION WITH FAB AND ESI

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With the increased emphasis in mass spectrometry on achieving lower detection limits and the need for analyzing increasingly smaller amounts of sample, usually as complex mixtures, suitable separation techniques must be devised for handling low levels of analytes in conjunction with mass spectrometric detection. We have been developing and applying two such methods which can be described as nanoscale separation techniques because of the low flow rates involved, i.e., nanoliters per minute. These are nanoscale capillary liquid chromatography (nCLC), with column ids of 50 to 75 μ packed with 5 or 10 μ particles, and capillary zone electrophoresis (CZE). Both separation techniques are characterized by high separation efficiencies and, in conjunction with mass spectrometry, high sensitivities. We have interfaced both of these separation techniques with continuous flow fast atom bombardment (CF-FAB) (using a coaxial interface) (1,2) on a VG ZAB 4F tandem mass spectrometer and with electrospray ionization (ESI) mass spectrometry on a VG 12-250 quadrupole mass spectrometer using a Vestec electrospray source.

A mixture of five peptides (Lys'bradykinin, Leu-enkephalin, proctolin, FLEEI, and MLF) was used to compare the various combinations of the four techniques. Fig. 1 a & b shows the separations obtained using nCLC (2 m x 75 μ id column packed to a depth of 25 cm with 10 μ particles) and CZE interfaced with coaxial CF-FAB. Fig 2 shows the same separations with ESI/MS detection. The table gives approximate detection limits for these compounds under the four conditions.

Several points can be gleaned from these data. A major point is that detection limits for the lower molecular weight peptides by CF-FAB on a magnetic sector instrument are better than for ESI analyses on a quadrupole. Given the better sensitivity of magnetic sectors over quadrupoles, this is not unexpected. Another point is that detection limits by CZE are approximately 40 times lower than are the detection limits obtained using nCLC (with the identical ionization technique). This is due to the higher separation efficiency inherent in CZE which leads to a greater flux of analyte per unit time. The downside of this increased efficiency and, thus, narrower peaks, is that, near the detection limits, the peaks are so narrow that magnet scans over a wide mass range are impractical. Thus, lys-bradykinin was out of the scan range used for the CZE experiments on the magnetic sector instrument. The third significant point is that the migration order is different for nCLC and CZE, because separation is based on different molecular characteristics in the two techniques. Thus nCLC and CZE represent two complementary separation techniques rather than competitive techniques.

References
ON-LINE NANOSCALE LIMITS OF DETECTION

Peptides by nCLC and CZE

<table>
<thead>
<tr>
<th>Analyte</th>
<th>nCLC CF-FAB</th>
<th>CZE CF-FAB</th>
<th>nCLC-ESI&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>Lys-Bradykinin</td>
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<td>a</td>
<td>45 pm</td>
<td>&gt; 10 pm</td>
</tr>
<tr>
<td>FLEEI</td>
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a) not analyzed
b) peak width 1.5
MICRODIALYSIS / MS: ON-LINE COUPLING OF BIOLOGICAL SYSTEMS WITH MASS SPECTROMETRY

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We have recently shown that coaxial CF-FAB is a very useful technique for the analysis of biomolecules (1). Due to the ability of the nanoscale capillary LC system to deliver a high concentration of analyte in a short period of time, this coaxial CF-FAB design allows for increased sensitivities over other CF-FAB interfaces. Detection limits of 500 attomoles in the multichannel acquisition mode and 1.8 femtomoles in the scanning acquisition mode have been obtained for a tripeptide. These detection levels are comparable to levels of some biochemicals in the body. This sensitivity, therefore, has prompted us to investigate the coupling of microdialysis with coaxial continuous flow FAB. Caprioll et al. first coupled microdialysis with CF-FAB for the analysis of drug levels in blood (2). We have recently coupled microdialysis with coaxial continuous flow FAB and tandem mass spectrometry (3). The combination of these techniques allows for the possibility of determination in real time of many biochemicals in the body.

The present investigation involves the determination of the half-life of a tris organophosphate, tris (2-chloroethyl) phosphate (TRCP), in rats. Several of these tris organophosphates have been widely used in plastics and synthetic fibers as flame retardants and are suspected carcinogens. The perfusion liquid is pumped at a flow rate of 0.8 μL/min through the dialysis probe. The perfusion liquid is contained in a stainless steel reservoir under helium pressure. The flow rate through the dialysis probe can be varied by varying the helium pressure. A sample loop of 1.1 μL is used to collect the perfusate. Injections are then made using a Valco injection valve and by using a static-split injection as previously described. Using this injection system (Figure 1), injections can be made at 2 minute intervals. The microdialysis probe was inserted inside a cannula surgically placed in the jugular vein of a 200-g male rat. In our initial in vivo microdialysis/MS experiments, TRCP was observed, however, background interferences from the FAB matrix occurred. To remove the background interferences, we integrated tandem mass spectrometry (MS/MS) into the protocol. In the MS/MS experiments, MS-I was set to pass only the protonated molecular ion of TRCP. This ion was forced to fragment by collisional activation and the most prominent fragment ion was monitored by MS-II to determine the level of the TRCP in the blood of the rat. After injecting 33 pm of the standard TRCP, the animal was connected to the injection system and was given a one-time dose of 20 mg/kg of TRCP injected into the femoral artery. The fragment ion of m/z 223 of TRCP was monitored for 60 minutes. The resulting chromatogram trace is shown in Figure 2. Traditional pharmacokinetic studies were also performed on rats using 14C-TRCP where blood samples were drawn and analyzed by HPLC. Both the mass spectrometric analyses and the HPLC analyses demonstrate that the pharmacokinetics of the TRCP follow a two compartment model in the rats (Figure 3A and 3B, respectively). Two half-lives, therefore, exist for the TRCP in the blood of rats, an alpha half-life due to distribution and elimination in the body and a beta half-life due to elimination only in the body. The alpha half-life determined by the mass spectrometric analyses is 2.9 minutes in comparison to 2.0 minutes as determined by HPLC. The beta half-life from the mass spectrometric analyses is 23.1 minutes, while that for the HPLC analyses is 32.7 minutes. A T-test of these results reveal no statistical difference between the results from the mass spectrometry analyses and the HPLC analyses.

References:
DNA-Adduct Analysis by CZE-FAB/MS

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A DNA adduct results when a chemical molecule covalently bonds to a purine or pyrimidine base of DNA. Such adducts can sometimes be removed by DNA repair mechanisms; however, analysis of existing DNA adducts provides information about both adduct persistence and the extent of exposure to genotoxic chemicals. DNA adducts can cause irreversible cellular changes that may lead to many different diseases. In some specific tissues and/or organisms, carcinogenesis may result. DNA adducts are important as Bioarkers; i.e. indicators of cellular damage that provide additional information about the future viability of a chosen tissue or organism. Thus DNA adducts may have an important role in Risk Assessment.

This preliminary research will show that a combination of Capillary Zone Electrophoresis (CZE) with flow Fast Atom Bombardment MS (FAB/MS) provides an important new mode of analysis of DNA adducts. The current post-labeling methodology is able to detect extremely low levels of adducts, but isn’t capable of providing the high degree of identification produced by MS.

The CZE (Model 3400, Applied Analytical Technology) was equipped with a 60cm, 75µm i.d. uncoated fused silica column (Polymicro Technologies) and a buffer of 0.1M pH9.6 Ammonium Acetate (Sodium [Bi]carbonate for UV analyses). The 10000V (10µA) operating voltage produced a 0.4µl/min electroosmotic flow directed toward the tail (cathode), and in less than 15 minutes resulted in excellent separations of unadducted deoxy-nucleosides and nucleotides. This performance was monitored by UV absorption with a variable wavelength UV detector (Linear Model 200) located 5cm from the tail of the column. Unadducted nucleosides eluted between 6.3 and 8 minutes, while the more negatively charged nucleotides eluted between 11 and 13 minutes. The UV detector was used both to develop CZE conditions and to establish the performance of the interface. In addition, since injections of as little as 5pg of adduct can be detected, the UV system can operated to screen samples for later confirmation by MS. Routinely, manual siphon injections are made with a height differential of 3cm for 10 seconds.

In Figure 2, the elution of both deoxy-nucleoside and nucleotide Guanine-Benz(a)pyrene adducts (Guanine is modified at the N2 position) is shown by UV absorption at 279nm. In each case the more massive adducts are less able to oppose the electroosmotic flow and thus elute earlier than would their unadducted counterparts. Note that in addition to the primary adducts, additional, as yet unidentified, nucleoside adducts elute in the intermediate region.
Both positive ion and negative ion full scan (100-600 daltons) spectra were obtained for as little as 7.5 ng of an acetylaminofluorene-guanine (AAF-G) adduct (guanine is modified at the C8 position). For a representative 15 ng positive ion analysis, Figure 3 shows an extracted ion current profile of mass 373, which corresponds to loss of the sugar from the molecular ion. The 35 second wide peak enabled the collection of 6 good scans (4.3 sec/scan) during the elution of the AAF-G. The corresponding averaged and background subtracted spectrum is shown in Figure 4. Note that the [M+H]$^+$ ion is relatively weak. The m/z 330 ion represents the loss of the acetyl group from the 373 ion, while m/z 224 represents the protonated acetylamino-fluorene. The analogous negative ion spectrum (not shown) gives complementary information, since the [M-H]$^-$ ion is the base peak, and a m/z 205 ion provides information about the C8 linkage position.

Single reaction monitoring was used to monitor the positive ion transition from the [M+H]$^+$ 489 ion to the 373 ion. Replicate injections gave reasonable reproducibility at 75 pg.

Real world application of these techniques necessitates the ability to remove from the sample almost all the unadducted nucleosides/nucleotides, since they would be present at levels of $10^6$ to $10^{10}$ times those of the adducts, and would prohibit the necessary volume reduction steps currently employed. Thus samples contaminated with a very small amount of adduct were first filtered with a BioRad SM2 membrane which retained only the adducts. Collection of the adducts with a 50% methanol rinse, and subsequent volume reduction, resulted in samples that could be analyzed. UV traces at 279 nm and 344 nm have shown the capability to recover and detect adducts present at less than 1 in $10^9$ normal nucleotides from 10 mg of DNA.
A LIQUID MATRIX PLAYS A SIGNIFICANT ROLE ON THE NATURE OF FAB SPECTRUM

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University of Tennessee, Department of Neurology, Memphis, Tennessee 38163

A liquid matrix plays a critical role in FAB-MS analysis. A number of matrices are in current use.\(^1\) Bombardment of a sample/matrix mixture by a high energy fast atom beam results in sputtering of the preformed ions in the form of protonated or deprotonated molecules. Production of radical cations have also been reported.\(^2\) A liquid matrix helps in maintaining a persistent emission of those solute molecular ions. Essential characteristics of a FAB matrix are high viscosity, nonvolatility, chemical inertness, and good solvency. Acid/base and oxidizing/reducing nature of the matrix, surfactancy of the solute relative to the matrix, and solute-solvent interaction all have profound effect on the nature of FAB mass spectrum.

In this study, we have investigated the influence of several liquid matrices on the nature of FAB spectrum of peptides and anthracycline antibiotics. The role of a matrix in promoting cleavage of the sulfate ester bond is also discussed. The matrices studied include glycerol, thiglycerol, dithiothreitol (DTT), 3-nitrobenzyl alcohol (NBA), and sulfolane. Experiments were conducted by using a VG 70 E-HF double-focusing mass spectrometer outfitted with an Ion Tech B11NF saddle-field FAB gun and DEC PDP 11/24-based VG 11-250 M+ data system.

It was reported earlier that the C-terminally-extended enkephalin undergo extensive fragmentation to produce mainly the Z'-type C-terminus ions when desorbed from glycerol matrix.\(^3\) On the other hand, CAD of the FAB-desorbed MH\(^+\) ions of those peptides resulted in cleavage of the peptide amide bond to yield Y'-type C-terminus sequence ions.\(^4\) The Z-type ions were of low abundance. This contrasting behavior clearly suggests the role of a liquid matrix in directing fragmentation of the peptide backbone. Indeed, a detailed investigation of the FAB mass spectrum of two octapeptides methionine enkephalin (Met-enk)-Arg-Gly-Leu and Dynorphin-1\(_4\) (Table 1) obtained using the above matrices demonstrates that a liquid matrix has a profound influence on FAB mass spectrum. Compared to other matrices, the use of glycerol resulted in extensive fragmentation of those peptides. Also, the type of sequence ions formed using glycerol differed from other matrices; the Z'-type ions being the dominant C-terminus ions in glycerol, whereas, the Y'-type ions dominated in the spectra obtained using the other above-mentioned matrices.

The sulfate ester bond in sulfated-leucine enkephalin (LES) is the most labile as attested by the preponderance of the loss of neutral SO\(_3\) (80 u) from the FAB-desorbed MH\(^+\) ion to yield m/z 556.\(^5\) The extent of this reaction varies with the matrix used. The (MH-80)/MH\(^+\) in glycerol, thiglycerol, DTT, NBA, and sulfolane is 2.2, 6.0, 6.2, 1.3 and 9.3, respectively. Likewise, it was noted that N-trifluoroacetamide-

All of the above observations suggest that the MH\(^+\) ions of peptides are formed with different internal energies in different matrices. An explanation consistent with these results is that MH\(^+\) ion is desorbed from the matrix as an ion-dipole cluster. Evaporation of the solvent results in vibrational cooling. The internal energy of the nude MH\(^+\), thus formed, depends upon the number of the matrix molecules associated with the initially-desorbed cluster and the Ion-dipole bond strength. Further support in favor of this explanation is the fact that the (MH-80)/MH\(^+\) of LES increases with the increase in the solute:solvent mole ratio (Figure 1). Furthermore, the fragment ion yield, as noted for Met-enk (Figure 2), increases with the scan number (in other words on the FAB irradiation time). With time, evaporation of the solvent on the probe increases the solute:solvent mole ratio.

The dominance of Z'-type C-terminus ions in the FAB spectra of the C-terminally-extended enkephalin when glycerol is used as the FAB matrix is intriguing. It was observed by us that the initially-formed ion-dipole complex of those peptides with glycerol undergoes further losses of H\(_2\)O, CH\(_3\)OH, and CH\(_2\)O to yield (MH+12)\(^+\) ion.\(^6\) It is our belief that that reaction promotes cleavage of the amino-alkyl bond to form the Z' ions. The experimental proof of this statement is the fact that FAB of Met-enk, which has poor facility for the (MH+12)\(^+\) ion formation, in glycerol yields both Y' and Z'-type sequence ions with equal ease, the Y' ions being more abundant (Table 3). In contrast, Met-enk-amide exhibits preponderance of Z' ions (Table 3), consistent with its higher propensity to undergo the (MH+12)\(^+\) ion formation. Furthermore, addition of 1 uL of 1% TFA to the Met-enk/glycerol and Met-enk-amide/glycerol mixtures results in lower abundance of Z' ions, whereas formation of the Y' ions is promoted (Table 3). This observation is parallel with the fact that addition of an acid drastically reduces formation of the (MH+12)\(^+\) ion in Met-enk-amide.

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In summary, vibrational cooling of the ion-dipole clusters occurs by evaporation of the solvent molecules. Thus, the ejected ions are formed with different internal energies, which depend upon the number of associated solvent molecules and the strength of the solvent-solute bond. Thus, the use of different matrices produces sample ions of different internal energies, with the consequence that the spectrum obtained differs from matrix to matrix.

REFERENCES

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Figure 1

Figure 2

Table 3

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The transparency of the lens depends on the highly structured aggregation and packing of the structural lens proteins, called crystallins. It is believed that posttranslational modifications of the lens proteins interfere with normal close packing and lead to cataracts. Bovine lens proteins comprise α, βH, βL and γ-crystallins, which can be separated by gel filtration chromatography. After further separation by reversed phase HPLC, the homogeneity of the β-crystallins was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrospray ionization mass spectrometry (ESIMS). Both methods showed that mixtures of proteins, and possibly different aggregates or degradation products, were still present after HPLC separation. While SDS-PAGE and ESIMS gave qualitatively similar results in terms of the number and molecular weight range of the components present in the β-crystallins, ESIMS gave more accurate molecular weights. For βB2, the major component of β crystallins which elutes from both βH and βL as the first HPLC peak, ESIMS gave a molecular weight of 23,215, which is only 6 Da greater than the value calculated from the cDNA determined sequence. Comparison of the ESIMS molecular weight for other β crystallins with the molecular weights expected for the gene products indicated that many modifications were present in the 2 year old bovine lens.

The primary sequence of βB2 was studied after digestion by trypsin by both conventional and continuous-flow FABMS. The continuous-flow system used (Fig. 1) consists of a gradient HPLC pump system, 2 sample injectors, a 1 mm ID microbore reversed phase HPLC column coupled to a CF-FAB probe via a fused silica capillary. The first injector was used to inject the whole peptide mixture formed from the enzymatic digestion of βB2. The second injector was used for direct injection of peptides previously separated by reversed phase HPLC or for the injection of a standard peptide to tune and check the calibration of the mass spectrometer. Post-column splitting from 50 μL/min to approximately 3 μL/min as the effluent enters the mass spectrometer was used.

Five nmoles of a tryptic digest of βB2 was injected and analyzed by continuous-flow (CF) FABMS. A gradient of 0-60% acetonitrile in water with 5% of glycerol, 3% of thioglycerol and 0.1% TFA over 30 minutes was used. Fig. 2 shows the selected ion chromatogram for the analysis. Several masses corresponding to peptides from βB2 are displayed. The spectrum of one scan of the analysis, scan 18, shows the presence of many peptides in a single scan (Fig. 3). The low chemical background of the CF-FABMS analysis makes detection of weak signals possible with less suppression from other species present. The peptides detected in this scan correspond to residues 108-111 (m/z 509), 68-75 (m/z 983), 90-100 (m/z 1216), 89-100 (m/z 1372) and 172-187 (m/z 1687) of βB2. Continuous-flow FABMS, supplemented by conventional FABMS, detected all but one of the peptides expected from a tryptic digest of βB2. The acetylated N-terminus was also confirmed. These results suggest that the proposed sequence for βB2 is probably correct.

Continuous-flow FABMS proved to be faster and gave better quality spectra than conventional FABMS for the analysis of lens crystallins. Excluding the HPLC separation and drying of each peptide fraction collected, conventional FABMS analysis of the βB2 digest took six hours. CF-FABMS analysis after an initial HPLC desalting step was completed in about 30 minutes. CF-FABMS gave better detection of very hydrophilic peptides which were difficult to detect by conventional FABMS. Continuous-flow FABMS will be a useful method for studying structures and identifying posttranslational modifications of lens proteins which may be involved in cataractogenesis.
Figure 1: Continuous-flow FABMS system used for analysis of a tryptic digest of PB2.

Figure 2: Selected ion chromatogram of some of the peptides expected to be found in a tryptic digest of PB2.

Figure 3: Mass spectrum of scan 18 of the continuous-flow FABMS analysis of PB2 tryptic peptides showing the presence of several peptides. The peak at m/z 509 corresponds to residues 108-111, m/z 983 is 68-75, m/z 1216 is from 90-100, m/z 1372 is 89-100, and the peak at m/z 1687 is residues 172-187. CF-FABMS allowed the detection of several peptides simultaneously so that optimum chromatographic resolution was not required.
CONTINUOUS FLOW-FAB MASS SPECTROMETRY OF HIGH MASS PEPTIDES

G. D. Roberts, W. P. Johnson, M. E. Hemling and S. A. Carr
SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406

Previously we reported the successful implementation of on-line continuous flow fast atom bombardment mass spectrometry (CF-FABMS) on our VG ZAB-SE 4F double focusing tandem mass spectrometer and described a number of its features in detail (1). One disturbing conclusion of this study was that the response by CF-FABMS for relatively large (M_r > 3000) peptides was significantly lower than could be obtained by standard probe FABMS in an optimized matrix. This trend was counter to what we expected based on the relative response of smaller peptides (M_r < 2000) in CF-FABMS vs. standard probe FABMS.

In order to gain a more thorough understanding of this phenomena and to improve CF-FABMS sensitivity at higher masses, a systematic study of four large peptides (C-peptide, [Tyr]-C-peptide, glucagon and Insulin B chain, oxidized) using different matrices (glycerol and thioglycerol) and probe tip geometries (domed tip vs. a domed tip with a flattened area ca. 1 mm in diameter centered on the fluid exit) has been conducted. Under static conditions the peptides responded similarly using the flattened domed tip vs. the standard flat ribbon tip; under these same conditions the original domed tip gave much poorer sensitivity. Under flow injection conditions thioglycerol gave a better response than glycerol by factors of 7 to 9, while the flattened domed tip gave ca. a factor of four improvement relative to the domed tip. Thus, an overall improvement of 30 to 35 fold in sensitivity thioglycerol gave a better response than glycerol by factors of 7 to 9, while the flattened domed tip gave ca. a factor of four improvement relative to the domed tip. Thus, an overall improvement of 30 to 35 fold in sensitivity.

To check the reproducibility of these results a second study was conducted in a similar manner. This study included the four high mass peptides used earlier as well as two low mass peptides, Ac-RGVGGLGLGK-NH_2 (Pierce S4, M 903) and des-Arg-bradykinin (M 954). We again found that thioglycerol was superior to glycerol as the matrix for flow FABMS of the high mass peptides, this time by factors of about 2 to 8 (Fig. 2). As for the lower mass peptides, des-Arg-bradykinin responds best in glycerol and Pierce S4 in thioglycerol with the flattened domed tip. Most importantly, the response for each of the high mass peptides by standard FABMS in an optimized matrix is from 15 to 100 greater than the highest response obtained by CF-FABMS. Also as noted previously the difference in the response of the low mass peptides between CF-FABMS and standard FABMS is much less than for the high mass peptides (Fig. 3). Probe geometry may be less important than originally determined, since in this second set of experiments the response of the high mass peptides with each probe tip is very similar. (Fig. 1).

The variability in the results (particularly those involving probe geometry) indicates that other factors must be controlled when conducting CF-FABMS. An examination of the system used to conduct the later study indicated that one obvious difference was the probe position in the source. During the earlier study the probe was in a more forward position, extending several millimeters beyond the front of the source block. This position was chosen because it had resulted in the greatest sensitivity. Between the time of the first study and the second, repeat study, the source and cesium gun were disassembled, cleaned and reinstalled. At the time the second study was conducted the optimal probe position for greatest sensitivity had changed, and was now several millimeters back with the tip actually inside the source block. Although the response by CF-FABMS was reasonable with the probe in this back position, instability and arcing were frequently problems, probably due to poor thermal contact with the block as freezing of the eluent was often evident. The probe could not be operated in the full forward position due to low sensitivity. Thus, a compromise position was used which turned out to be about midway between the two extremes. In this position stability was achieved and the response could be made reasonable by repositioning the cesium ion gun. The repeat study was conducted using the probe in this position.

In conclusion, thioglycerol should be chosen over glycerol for high mass peptide analysis by CF-FABMS. It provides an improvement in response by factors of at least 2 to 8 and perhaps more when all conditions are optimized. Probe geometries (domed tip vs. flattened domed tip) may not be as critical. However, under certain conditions the flattened domed tip may offer an advantage by as much as a factor of 4. For the best response of high mass peptides standard FABMS should be chosen over CF-FABMS because even the greatest response of any of the four peptides by CF-FABMS is several orders of magnitude less than that of standard FABMS. Finally it should be emphasized that CF-FABMS is a complex technique whose success depends on a number of potentially critical, interrelated factors, not simply on one or two independent parameters such as probe tip geometry and matrix. The probe position appears to be among these factors, but it may not be intrinsically the critical factor; this remains to be explored. The x-position does affect probe heating efficiency, which in turn affects probe temperature (an important factor since it affects signal response and stability) due to its relationship with the source block.

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Figures 1, 2, and 3 show various comparisons:

- **Figure 1** compares Domed Tip vs. Flattened Domed Tip and Glycerol vs. Thiglycerol.
- **Figure 2** shows CF-FAB vs. Standard FAB.

The figures illustrate the differences in mass spectrometry performance under these conditions.
SEPARATION AND IDENTIFICATION OF CAROTENOIDS USING CONTINUOUS-FLOW FAST ATOM BOMBARDMENT LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY.

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Of the numerous plant carotenoids in the human diet, more than 50 possess provitamin A activity (1). In addition, certain carotenoids have received considerable attention as having a possible role in the prevention and treatment of cancer (1). Approximately 600 different carotenoids have been characterized, many similar in structure, and for each of these compounds, there may exist a number of geometric and optical isomers (2). The similarity of physical properties within this group of compounds poses an analytical challenge in resolving and isolating individual components for structure elucidation.

Currently, high performance liquid chromatography (HPLC) is regarded as the method of choice for the separation, identification, and quantitation of carotenoids found in biological tissues. Although HPLC is well suited for carotenoid analysis, complex mixtures of these compounds often found in biological tissues can be difficult to resolve and identify with confidence. A minimum of identical retention time and spectrophotometric characteristics with known standards are necessary for tentative identification of carotenoids, although these two parameters alone are not sufficient for positive identification (3). Obtaining a mass spectrum of an individual carotenoid in conjunction with accurate spectrophotometric and chromatographic data is sufficient to identify the carotenoid. However, additional analytical data are required to confirm the identities of stereoisomers.

Approximately 40 g of carrots or tomatoes were pureed, filtered, washed with methanol, and then extracted using 100 ml of hexane under nitrogen. The hexane extract was filtered again, concentrated to dryness under a stream of nitrogen, and stored at -20°C until analysis. The sample was reconstituted in the HPLC mobile to form a solution of approximately 1 µg/µl. All samples were handled under yellow lighting.

HPLC separations were carried out using an Applied Biosystems Model 140A dual syringe pump, Vydac C18 HPLC column (2.1 mm x 15 cm), and Applied Biosystems Model 757 UV/VIS absorbance detector set at 450 nm. The solvent system consisted of isocratic ethyl acetate/acetonitrile/methanol (20:50:30; v/v/v) or a 20 min gradient from 50/50/0/0.5 to 10/30/60/0.5 (v/v/v/w) water/methanol/ethyl acetate/glycerol. The flow rate was 100 µl/min. The eluate from the HPLC column was split so that approximately 7 µl/min flowed to the mass spectrometer. Mixtures containing approximately 5 µg of the most abundant carotenoid component were injected onto the HPLC column per analysis. Because of the solvent split, approximately 0.5 µg of each carotenoid entered the continuous-flow FAB interface. For continuous-flow FAB LC/MS, aqueous 1% 3-nitrobenzylalcohol was added to the mobile phase post-column at 1.4 µl/min. Alternatively, glycerol was added directly to the mobile phase.

FAB and continuous-flow FAB mass spectra were obtained using a JEOL HX110HF double focusing mass spectrometer equipped with a DA5000 data system. B/E linked scans were carried out following collisional activation and 5 to 8 scans were accumulated per analysis. The helium pressure in the collision cell in the first field-free region was adjusted so that the signal was attenuated 70%.

Initially, isocratic HPLC with absorbance detection without mass spectrometry was used to isolate α-carotene, β-carotene, and lycopene from the tomato extract. The isolated carotenoids were then analyzed using static positive ion FAB with 3-nitrobenzylalcohol as the matrix. Molecular ions, M+, were detected for each of these carotenoids at m/z 536. B/E linked scanning was then used with collisional activation to detect fragment ions of each of these isobaric carotenoids. Because these three carotenoids were structural isomers, fragment ions were detected which were unique to each compound (data not shown).

Next, the HPLC methods described above were used on-line with continuous-flow FAB mass spectrometry for analysis of a standard mixture of carotenoids containing α-carotene, β-carotene, and lutein. Using the gradient HPLC solvent system, the protonated molecule of lutein at m/z 569 was observed, but no ions of α-carotene, β-carotene were detected. Therefore, 3-nitrobenzylalcohol was investigated as an alternate FAB matrix. Addition of aqueous 3-nitrobenzylalcohol to the mobile phase facilitated the formation of abundant molecular ions, M+, for all carotenoids investigated. However, the chromatographic resolution was compromised. Therefore, aqueous 3-nitrobenzylalcohol was added post-column.
By using isocratic organic solvents with post-column addition of aqueous 3-nitrobenzylalcohol, a stable continuous-flow FAB signal was obtained, and molecular ions could be detected for all carotenoids investigated. For example, the total ion chromatogram (TIC) and several selected ion chromatograms for the LC/MS analysis of the carrot extract are shown in Figure 1. The five major carotenoids species in carrots were at least partially resolved from each other by reversed phase HPLC and could be easily identified by their molecular ions in the continuous-flow mass spectra. In Figure 2, the positive ion FAB mass spectrum of β-carotene acquired during LC/MS (retention time 10 min) is presented.

Figure 1. Positive ion continuous-flow FAB LC/MS analysis of carotenoids extracted from carrots showing the reconstructed total ion chromatogram (TIC) and selected ion chromatograms for the molecular ions of the five most abundant carotenoids.

Figure 2. Positive ion continuous-flow FAB mass spectrum of β-carotene acquired during the LC/MS analysis shown in Figure 1.

Isocratic reversed phase HPLC followed by post-column addition of FAB matrix, on-line absorbance detection, and on-line continuous-flow LC/MS analysis constitutes a rapid, sensitive, and specific technique for the identification of carotenoids extracted from foods. This method is the first continuous-flow FAB LC/MS method reported for carotenoid analysis. Studies are in progress to improve the sensitivity of the method by using microbore HPLC without solvent splitting.

UTILITY OF LC/FAB AND THERMOSPRAY MASS SPECTROMETRY FOR STRUCTURE ELUCIDATION OF METABOLITES FROM A FLORINATED CHLOROACETANILIDE HERBICIDE

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We have recently established an on-line microbore HPLC/FAB technique on a VG-ZAB HF mass spectrometer for identification of polar metabolites from herbicides and fungicides in Monsanto. This technique can now be used routinely for identification of polar metabolites with good chromatographic resolution and excellent FAB sensitivity with strong pseudomolecular ions. Scanning high-resolution LC/FAB mass spectrometry can also provide molecular formulae for structural elucidation of unknown metabolites. On the other hand, identification of less polar metabolites can still be carried out by the thermospray (TSP) method using a Finnigan quadrupole mass spectrometer. The thermospray LC/MS method provides useful structural information for both polar and less polar metabolites because their spectra show more fragmentation (pseudomolecular ions of polar metabolites are sometimes not seen) than FAB spectra. Both LC/FAB and LC/TSP techniques complement each other for analysis of metabolites of agrochemicals, since LC/FAB is generally suited to polar metabolites while thermospray LC/MS is good for less polar compounds.

The in vitro metabolism of herbicides by rat liver enzyme preparations can provide important information of the types of metabolites that may be produced during subsequent in vivo metabolism studies. Such information is obtained rapidly since in vitro incubations can usually be completed within a few hours. These LC/MS methods are ideally suited for in vitro studies since sample purification and concentration are not essential at all.

As an example, the in vitro metabolism study of an experimental Monsanto herbicide, 2-chloro-N-(ethoxymethyl)-N-[2-methyl-6-(trifluoromethyl)phenyl] acetamide, was carried out using S-9 rat liver enzymes in the presence of NADPH and glutathione.

Radiolabeled substrate was used in the incubations to allow the progress of metabolism to be monitored by HPLC and radioactivity detection (HPLC/RAD). To facilitate identification, the substrate also contained an appropriate amount of 13C-labeled material to provide a 12C/13C "doublet" in the mass spectra of metabolites. Glutathione conjugation was the major reaction observed during in vitro incubation of the herbicide. Oxidation of the chloroacetanilide by rat liver enzymes was also a significant metabolic reaction. Seven metabolites were identified, of which four were glutathione conjugates (Table 1).
Table 1

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (substrate)</td>
<td>H</td>
<td>CH₂OEt</td>
<td>Cl</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>H</td>
<td>C</td>
</tr>
<tr>
<td>3</td>
<td>OH</td>
<td>CH₂OEt</td>
<td>Cl</td>
</tr>
<tr>
<td>4</td>
<td>OH</td>
<td>H</td>
<td>Cl</td>
</tr>
<tr>
<td>5</td>
<td>H</td>
<td>CH₂OEt</td>
<td>glutathione</td>
</tr>
<tr>
<td>6</td>
<td>H</td>
<td>H</td>
<td>glutathione</td>
</tr>
<tr>
<td>7</td>
<td>OH</td>
<td>CH₂OEt</td>
<td>glutathione</td>
</tr>
<tr>
<td>8</td>
<td>OH</td>
<td>H</td>
<td>glutathione</td>
</tr>
<tr>
<td>9 *</td>
<td>H</td>
<td>CH₂OH</td>
<td>Cl</td>
</tr>
</tbody>
</table>

* very unstable and instantaneously converted into 2.
Analysis of Bovine β-Casein Tryptic Digest by Continuous Flow Fast atom Bombardment Mass Spectrometry

D.S. Jones, G.A. Warburton, H. Wight, J. Moncur and C. Moore
Kratos Analytical, Barton Dock Road, Urmston, Manchester, M31 2LD, UK

Experimental

The study was performed using a CONCEPT I H mass spectrometer operating at 2000 resolution (10% valley). Accelerating voltage was maintained at 8 kV and the magnet was scanned from mass 3500 to 400, and in subsequent experiments, 2750 to 50, at 10 sec/decade. A standard FAB gun was used to produce an 8 kV primary xenon atom beam. The most stable conditions were observed at a source temperature of 70°C and source pressure of 2 x 10^{-4} Torr. Acquired centroided data were processed using a Kratos MACH 3 data system.

For the LC system, Solvent A was 0.06% trifluoroacetic acid (TFA) in water and Solvent B was 0.06% TFA in 80% acetonitrile 20% water. The gradient employed was 100% A, isocratic for 5 minutes, followed by a linear gradient to 100% B over the next 5 minutes. The column used was ODS1 Spherisorb (2 mm x 150 mm) with a flow rate of 0.2 ml per min. The injection volume was 20 μl which corresponds to 20 ng of the total digest. A further 0.2 ml per min. of 2% glycerol in acetonitrile was added post-column to provide a FAB matrix. A standard splitting device maintained a constant flow-rate to the mass spectrometer of about 5 μl per min., using a split ratio of 80:1. A schematic diagram of the liquid chromatograph introduction system is given in Figure 1.

Results

The identified peptide fragments have been matched to the relevant portion of the β-casein molecule (Table 1). There is a considerable amount of redundancy in these data as many of the peptides originate from overlapping sequences. However, it has still been possible to map 60% of the total protein.

Table 1: Amino Acid Sequence of Bovine β-Casein A² Variant.

<table>
<thead>
<tr>
<th>Residue</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>209</th>
</tr>
</thead>
<tbody>
<tr>
<td>REEELNVPGEIEVLZLZEESETIR/KKKIEKFOZIZQQQTEDELOKKH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
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<td></td>
<td></td>
<td>150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VGPFPFPW</td>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

(z, denotes phosphoserine residue) (highlighted sequences verified by this study)
A total of 25 peptides were observed in the investigation and good correlation was obtained between the UV and the total ion chromatogram. Table 2 lists identified components, protonated molecule ions and chemical origin (in elution-time order) of 20 of the peptides expected from this analysis. Figure 2 shows the ion chromatograms from some protonated molecular ions. These peptides can all be explained as arising from either tryptic and/or chymotryptic action, indicating that the trypsin used was not completely free of chymotryptic activity. The FAB spectra were used to interpret peptide sequences. Figure 3 shows a typical peptide spectrum, in this case of component X.

**Table 2: Identified Peptide Fragments**

<table>
<thead>
<tr>
<th>Code</th>
<th>Residues</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>106-107</td>
<td>HK</td>
</tr>
<tr>
<td>B</td>
<td>99-99</td>
<td>VE</td>
</tr>
<tr>
<td>C</td>
<td>26-29</td>
<td>INIK</td>
</tr>
<tr>
<td>D</td>
<td>26-29</td>
<td>INOX</td>
</tr>
<tr>
<td>E</td>
<td>30-33</td>
<td>EK</td>
</tr>
<tr>
<td>F</td>
<td>164-169</td>
<td>SLEIQK</td>
</tr>
<tr>
<td>G</td>
<td>29-32</td>
<td>KIE</td>
</tr>
<tr>
<td>H</td>
<td>100-105</td>
<td>EAAAPK</td>
</tr>
<tr>
<td>I</td>
<td>33-48</td>
<td>FZKEQGGIEQTEDELDK</td>
</tr>
<tr>
<td>J</td>
<td>177-183</td>
<td>AYPPQR</td>
</tr>
<tr>
<td>K</td>
<td>172-176</td>
<td>VPPQK</td>
</tr>
<tr>
<td>L</td>
<td>191-193</td>
<td>LLY</td>
</tr>
<tr>
<td>M</td>
<td>194-202</td>
<td>EGVYGPVR</td>
</tr>
<tr>
<td>N</td>
<td>106-111</td>
<td>HKEMPP</td>
</tr>
<tr>
<td>O</td>
<td>106-113</td>
<td>EMPPK</td>
</tr>
<tr>
<td>P</td>
<td>49-52</td>
<td>HPP</td>
</tr>
<tr>
<td>Q</td>
<td>184-190</td>
<td>DNVPQAF</td>
</tr>
<tr>
<td>R</td>
<td>33-52</td>
<td>FZKEQGGIEQTEDELDK</td>
</tr>
<tr>
<td>S</td>
<td>191-202</td>
<td>LVEQPGVPVR</td>
</tr>
<tr>
<td>T</td>
<td>203-209</td>
<td>GDPRK</td>
</tr>
</tbody>
</table>

- = due to tryptic action
- = due to chymotryptic action
[=] = suggested non-Cerebrinus peptide

**Conclusions**

The combination of HPLC and continuous flow FAB MS is capable of providing a wealth of useful information to the peptide chemist. Complex mixtures are analysed speedily, and with moderate ease, without resorting to off-line separation of components or derivatization procedures. Results indicate that chromatographic integrity is adequately maintained during analysis.
Applications of a Fast Neutral Beam Ion Source Coupled to an FT/ICR Mass Spectrometer

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The sensitivity, wide mass range, high resolving power, and versatility of a fast neutral beam ion source coupled to a Fourier transform ion cyclotron resonance (FT/ICR) mass spectrometer have been demonstrated. The fast neutral beam ion source was originally developed by Delmore [1]. A beam of SFe⁻ ions is formed, focused, and accelerated with an energy of 5-10 keV down the bore of a superconducting magnet. Because the SFe⁻ ions are formed in short-lived excited quantum states, autoneutralization occurs, producing a beam of focused neutral SF₆ molecules which passes unaffected through the fringe field of the magnet. Undissociated ions and electrons may be removed from the beam by a charged deflector plate situated close to the superconducting magnet. The neutral beam impacts a sample placed on a solids insertion probe, and the desorbed ions are continuously trapped and then pulse-excited and -detected in a cubic ion trap. Preliminary results of the feasibility of this ion source for FT/ICR mass spectrometry were presented at the 1990 ASMS meeting [2]. Delmore and Appelhans have previously demonstrated the efficacy of the neutral beam as an ionization source for a quadrupole mass spectrometer [3], in which ions were detected up to the 350 u/e upper mass limit of the quadrupole.

We have now demonstrated the feasibility of the experiment (for both broad-band and high-resolution FT/ICR positive-ion mass spectra) with tetra-butylammonium bromide and (see Fig. 1) Tylenol®. The sensitivity of the new hybrid technique is vastly superior to static SIMS for electrically insulating samples, as demonstrated by a mass spectrum (not shown) of Teflon® extending up to m/z = 600 u/e [C₁₂F₂₄⁺]. The ability to analyze samples without the need for a matrix has been demonstrated with methyl stearate. The present results have been compared to those obtained by: (a) a fast neutral beam ion source coupled to a quadrupole mass spectrometer; (b) FAB on a double-focusing sector instrument; and (c) laser desorption on an FT/ICR mass spectrometer. All of the advantages of the fast neutral beam ion source previously demonstrated with quadrupole mass analysis are preserved, and the additional advantages of FT/ICR mass analysis (e.g., high mass resolving power, ion trapping) are retained. This work will be reported in fuller detail elsewhere [4].

Acknowledgements: The authors thank A. D. Appelhans, D. A. Dahl, and J. E. Delmore for the fast neutral SF₆ beam gun design, and for helpful advice and assistance. We also thank David Chang for providing FAB spectra. This work was supported by the National Science Foundation (CHE-8721498) and The Ohio State University.

Figure 1. Top: A broad-band FT/ICR mass spectrum of Tylenol® (top) obtained by use of a fast neutral beam ionization source. Bottom: high-resolution FT/ICR mass spectrum of the pseudomolecular (M+H)^+ ion.
The yield of desorbed ions in $^{252}$Cf-plasma desorption mass spectrometry is dependent upon the energy and incident angle of the fission fragment. A distribution of fission fragments, with respect to mass, energy and charge, is produced in the fission process. Asymmetric fission of the $^{252}$Cf nucleus results in the production of light and heavy ions; the "average" ions in these distributions correspond to species such as $^{106}$Mo$^{43}$ (104 MeV) and $^{142}$Ba$^{56}$ (79 MeV). The energy of these primary ions is reduced by 44 - 46 MeV after passing through the Ni foil covering the $^{252}$Cf source, the target backing and the sample itself. The ion yield for insulin is reduced by a factor of three as a result of this effect. The trajectory of the incident fission fragment also affects secondary ion yields; more oblique incident angles result in enhanced secondary ion yields. However, for $\theta \geq 80^\circ$, the light fission fragments cannot penetrate a typical sample film. To minimize these effects, the foil covering the $^{252}$Cf source was reduced from 1 $\mu$m Ni to 0.25 $\mu$m Ni. This factor accounts for almost half of the energy lost by the fission fragments. In addition, a new sample backing has been developed. This sample support is made by casting films of polyvinyl formal (Formvar) on glass slides. The films are floated off the slide and captured on a supporting ring. The films are coated with a thin (10 - 50 nm) layer of Au to provide a conducting surface. Foil thicknesses are accurately determined by measuring the energy loss spectrum of alpha particles penetrating the foil. These thin foils can be used in any application where Mylar films have been employed. For example, nitrocellulose can be electrospayed onto the surface for protein adsorption. In comparison to the 1.5 $\mu$m aluminized Mylar (polyethylene terephthalate) foils that are routinely used, the energy loss is reduced from $-13$ MeV to 1 MeV.

We have also investigated the formation of self-assembled mono- and multilayers on the Formvar films to produce surfaces with a variety of different functional properties. Self-assembled layers are formed as a result of strong specific interactions between the adsorbate and the surface resulting in the spontaneous adsorption of the reactive end of the adsorbate. The interactions may be based upon favorable electrostatic, hydrophobic and/or chemical reactions. For example, carboxylated surfaces may be prepared by constructing a cationic surfactant-fatty acid multilayer on the Formvar surface. To produce this surface, the Formvar is impregnated with tridodecylmethylammonium chloride (TDMAC). The resulting surface is nonelutable, much more hydrophilic than the Formvar surface and has enhanced binding capacities for anionic species. This surface therefore avidly binds fatty acids such as sodium stearate. If the fatty acid adsorbed with the carboxyl group oriented towards the quaternary ammonium surface, we would expect that the surface layer would consist of a
hydrophobic hydrocarbon layer. However, we have observed that this surface has a high affinity for cationic analytes and, is very hydrophilic. This can only be explained if, like the TDMAC, multilayers were adsorbed resulting in a surface layer enriched with carboxylic groups. A comparison of the positive ion spectra for CsI adsorbed onto TDMA-Formvar and CsI adsorbed onto stearate-TDMA-Formvar is shown below. The abundant iodide ion observed in the negative ion spectrum and the TDMA$_2$I$^+$/TDMA$^+_2$ clusters have been completely displaced by the stearate anion. Although the positive molecular ion of the stearate is obscured by the presence of TDMA fragment ions, the positive ion oligomers are apparent in the higher mass range. A prominent negative molecular ion of the stearate is present in the negative ion spectrum. This basic approach can be used to produce surfaces with a wide variety of functional groups which can be tailored to adsorb specific analytes.

This work was supported by grants from the National Institutes of Health (GM26096) and the Robert A. Welch Foundation (A-258).
Matrix-assisted UV laser desorption is recognised as being an extremely promising technique for the production of gaseous molecule-ions from thermally labile, non-volatile biomolecules of high masses. The presence of the matrix appears to change qualitatively the characteristics of the desorption process. In the absence of matrix, it was in our experience always difficult using laser desorption to produce molecule-ions from peptides with molecular masses much above 1000 Da. With a suitable matrix, it is possible to produce molecule-ions with masses above 100 000 Da. We have been interested in the role played by the matrix, and have investigated effects on the laser desorption mass spectra of varying the molar ratio of matrix to analyte. In the course of these investigations, we discovered that the concentration of the matrix/analyte solution loaded onto the probe (and dried before introduction into the mass spectrometer) had an effect on the mass spectrum, even though the ratio of matrix to analyte did not change. We have concluded that these effects are a consequence of differing crystal structures influencing the observed mass spectra.

The laser desorption mass spectra were measured using a time-of-flight system incorporated in the first field-free region of a large research mass spectrometer (MMM). The arrangement is designed to allow ions to be examined either by double-focussing mass spectrometry or by time-of-flight. This particular combination of magnetic sector and time-of-flight will be referred to as "parallel MAG-TOF". The laser employed is a dye laser pumped by an excimer laser. A wavelength of 266 nm has been used to irradiate the sample. In the case of nicotinic acid and insulin, we had found that at a molar ratio of about 600:1 the laser desorption mass spectra did not contain a strong peak attributable to nicotinic acid molecule-ions. This absence of matrix peaks was found at threshold irradiances and when using certain concentrations of the matrix/analyte mixed solution. The solutions were dried in a stream of nitrogen.

We report here that the macroscopic crystal structure of the sample (matrix/analyte) varies considerably, depending on the matrix/analyte ratio and on the drying procedure employed. Viewed under a low-power optical microscope, a nicotinic acid/insulin sample at a 600:1 ratio and dried under a stream of nitrogen (-200 cm\(^{-2}\) min\(^{-1}\)) has the appearance of a reasonably homogeneous crystalline material. Nicotinic acid/myoglobin at a ratio of 800:1 and dried with the aid of nitrogen has a similar appearance. When the molar ratio is higher (6000:1), samples of either nicotinic acid/insulin or nicotinic acid/myoglobin have a heterogeneous appearance, because the samples occur as discrete, larger crystals clearly separated from each other. The crystals are typically
about 10-50 \mu m in diameter in the case myoglobin. Lower molar ratios (60:1) give crystals of a homogeneous appearance. Pure nicotinic acid dries to give crystals with a voluminous, fluffy appearance.

It is possible to draw tentative correlations between the macroscopic crystal structure and the laser desorption mass spectra when using nicotinic acid as a matrix. The homogeneous (in appearance) samples at ratios of about 600:800 to 1 yield spectra at threshold irradiance, in which matrix peaks are very weak if not absent. There seems to be an optimum molar ratio, in terms of accentuating the protein molecule-ion at the expense of the matrix, which is about 600 for insulin and higher for myoglobin. The heterogeneous (in appearance) samples at ratios of about 6000:1 give spectra containing matrix peaks.

The same experiments with sinapinic acid as the matrix in place of nicotinic acid gave similar results as regards the microscopy. The apparently homogeneous samples did not, however, give spectra without matrix peaks. To date, we have not obtained any spectrum without peaks in the vicinity of the matrix mass when using sinapinic acid as a matrix.

In conclusion, we emphasise that we do not know whether or not the matrices and analytes are co-crystallising, i.e. whether mixed crystals are being formed under any of the conditions. Further experiments are required to confirm that co-crystallisation does occur. What is clear is that sample preparation is crucially important, and that the rate of drying of the sample is particularly critical. It also seems clear that subsequent to photon absorption there is a time interval prior to separation of isolated molecule-ions within which charge transfer processes can occur.

REFERENCES


Expansion Cooling in Matrix Assisted Laser Desorption (a Hydrodynamics Study)

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Matrix assisted laser desorption attracts considerable attention because of its capability to volatilize large molecules of biological interest. Mass spectrometry as well as other gas phase or vacuum spectroscopic and separation techniques can benefit from this progress.

One of the basic questions related to this new method concerns the mechanism of volatilization. More specifically we are interested in the nature and in the kinetics of the phase change induced by the laser pulse.

The one-dimensional hydrodynamic code developed for treating laser plasma ignition problems [1] has been adapted to this low irradiance case by taking more precise account of solid heating and phase transitions. In the calculations a matrix - vacuum interface was under the influence of a frequency quadrupled Nd-YAG laser with 10ns pulse length. The optical and thermal characteristics of the matrix were chosen close to those of nicotinic acid and of other good matrices. The model accounted for mass, momentum and energy transfer across the interface and for light absorption, heat conduction and phase transition inside the solid.

During the early period of the light pulse the solid is heated by about $10^6$K/s heating rate resulting in more and more vigorous evaporation through direct sublimation. By the end of the laser pulse a certain amount of matrix is evaporated (together with the embedded biomolecules) and the surface region of the solid is superheated (Fig. 1/a and 1/b; 10ns curves). Heat conduction and evaporation cools the surface quickly under the phase transition temperature after the laser pulse has ceased (50ns curve in Fig. 1/a). Fast expansion and translation of the plume towards the vacuum can be observed in Fig. 1/b and 1/c (50ns and 100ns curves). At the same time Fig. 1/a shows strong expansion cooling of the plume.

On the basis of these findings an interesting analogy is apparent between the matrix assisted method and the so called two-laser experiments. In these setups a separate laser is user for volatilization and ionization. The plume generated in the first step is quenched by a crossed expanding argon jet. In the matrix assisted laser desorption the expanding matrix plume acts as a “built in” jet intimately mixed with the guest molecules.

Several quantitative features of matrix assisted laser desorption experiments can be also rationalized by the heating of the solid phase and by plume hydrodynamics, as it is described by our model. Plume generation is bound to the condition that surface temperature exceeds the sublimation temperature of the matrix. The related threshold irradiance is below $10^7$W/cm$^2$; a value close to experimental observations. The mean velocity of the plume centre of mass gives an estimate of neutral particle energies in the order of ~60 meV, again close to measured figures. Furthermore, strong expansion cooling of the plume is observed down to around 100 K which may account for the stabilization of the large molecules and ions and for their appearance in the mass spectra.

The low thermal load on the embedded biomolecules during the volatilization process was demonstrated by our experiments using extremely thermolabile molecules as "molecular thermometers" [2]. The complete lack of thermal degradation processes was reflected by the mass spectra which also pointed to fast heating rates and to the existence of a stabilizing quenching mechanism.

The findings of this hydrodynamic study complement our earlier results which indicated an energy transfer bottleneck between the matrix and the embedded biomolecules [3]. According to this homogeneous bottleneck model the energy is deposited into the matrix molecules and the frequency mismatch between their intramolecular vibration and the vibration frequencies of the hydrogen bonds prevents the transfer of energy to the internal degrees of freedom of the embedded molecules. A combination of the two mechanisms (i.e. energy transfer bottleneck in the solid phase and expansion cooling in the gas phase) can give a full description of the volatilization mechanism.
Figure 1. The effect of a 10 ns frequency quadrupled Nd-YAG laser pulse ($10^7$ W/cm$^2$) on the matrix-vacuum interface. The spatial temperature (a/), density (b/) and velocity (c/) distributions are shown at different times. Surface position is marked by the vertical dashed line. In Figure 1/a the starting temperature is shown by the horizontal dashed line.

INVESTIGATION OF MATRIX-ASSISTED LASER DESORPTION FTMS FOR BIOMOLECULES

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Structural characterization of biomolecules at the isomeric level is necessary for determining oligonucleotide sequences and examining DNA modification induced by carcinogenic agents. Matrix-assisted laser desorption can be used with FTMS for the detailed structural investigation of non-volatile, polar biomolecules, such as normal and modified oligonucleotides. For these experiments, laser desorption using a nicotinic acid matrix and 266 nm radiation was found to generate informative negative ions from oligonucleotides, including abundant (M-H)^- ions which could be used for molecular weight determination. The accurate mass measurement and ion manipulation capabilities of FTMS were used to obtain molecular weight, sequence information (at the isomeric level), and the identity and location of adducts for small oligonucleotides.

Experimental parameters for the matrix-assisted LD-FTMS technique were optimized with oligonucleotide dimers.[1] The negative ion spectra for these compounds revealed not only molecular weight information from the (M-H)^- ions but also detailed sequence information from the fragment ions. Two major fragmentation pathways were observed; cleavage of the glycosidic bond between the nucleic base and the sugar ring, and cleavage of the phosphate linkage to eliminate primarily the 5'-nucleoside. This latter fragmentation pathway provided not only sequence information but also identification of the 3'-nucleotide, and enabled differentiation of isomers such as d(5'-GA-3') and d(5'-AG-3').

Oligonucleotide trimers could also be characterized by the matrix-assisted LD-FTMS technique. The nicotinic acid matrix was essential for the success of these experiments. Elimination of the matrix resulted in complete loss of the (M-H)^- ions as well as most of the larger fragment ions for these oligomers. The negative ion spectrum of d(TGT) revealed an (M-H)^- ion at m/z 874 along with fragment ions at m/z 650 and 321, which result from phosphate ester cleavage and provide sequence information. For this particular compound, it is impossible to unambiguously determine whether these fragment ions result from elimination of the 5' or 3' nucleosides due to the symmetry of the compound. Isolation and collisional dissociation of the (M-H)^- ion at m/z 874 for d(TGT) revealed identical fragmentation as observed in the laser desorption experiment (i.e., m/z 650 and 321 as abundant fragment ions), implying that the cleavage of the phosphate ester linkage is the primary fragmentation pathway for collisional dissociation as well.

The negative ion spectra for tetranucleotides revealed more extensive fragmentation than that observed for the smaller oligomers. For example, Figure 1 illustrates the negative ion spectrum for the tetramer d(AGCT), with a molecular weight of 1173. This spectrum was obtained by using 2-pyrazinocarboxylic acid as the laser matrix and 266 nm radiation. Note the (M-H)^- ion at m/z 1172 and the fragment ions at m/z 939, 739, 610, and 321. The fragment ions at m/z 939, 610, and 321 are "Y" [2] fragment ions corresponding to elimination of nucleotides from the 5' end of the oligomer. This particular fragmentation provides not only sequence information but also identifies the 3' end of the oligomer. The fragment ion at m/z 739 results from a two-bond cleavage, as shown in Figure 1, and has been observed in FAB spectra of other oligonucleotides.[3] In order to verify the fragmentation pathways, an isomeric tetramer d(TGCA) was examined. The abundant Y sequence ions for this oligomer were different from the tetramer d(AGCT), as shown in Table 1. Note that "X" ions (phosphate ester cleavage from the 3' end of the oligomer) are also observed but in lower abundance than the Y ions. The presence of modifications such as alkyl adducts on these small oligomers could also be identified. For example, the fragment ions observed in the negative ion spectrum of a tetramer containing guanine alkylation provided the information necessary to determine the site of methyl attachment in the oligomer, shown in Table 1.

Extension of matrix-assisted laser desorption FTMS to larger biomolecules requires additional examination of experimental parameters which may influence the accessible mass range for FTMS. In particular, the ability to trap and subsequently detect large ions generated by matrix-assisted laser desorption is under investigation. Because ions generated by the matrix-assisted LD technique can have substantial kinetic energy distributions, it is necessary to examine methods of cooling the ions in order to improve the trapping efficiencies.


### Table 1. Negative ions observed for tetranucleotides (266 nm matrix-assisted LD-FTMS)

<table>
<thead>
<tr>
<th>Tetramer</th>
<th>(M-H)</th>
<th>Sequence ions m/z (% relative abundance)</th>
<th>Y</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>d(AGCT)</td>
<td>1172</td>
<td>939 (7) 610 (25) 321 (100)</td>
<td>948 (-) 659 (4) 330 (9)</td>
<td></td>
</tr>
<tr>
<td>d(TGCA)</td>
<td>1172</td>
<td>948 (4) 619 (26) 330 (89)</td>
<td>939 (-) 650 (4) 321 (30)</td>
<td></td>
</tr>
<tr>
<td>D(TG'CA)</td>
<td>1186</td>
<td>962 (2) 619 (7) 330 (8)</td>
<td>953 (-) 663 (2) 321 (2)</td>
<td></td>
</tr>
</tbody>
</table>

G' = O6 - methyl guanine

---

Figure 1
Laser Desorption/Postionization Mass Spectrometry of Polymer Additives by TOF Analysis

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INTRODUCTION

The determination of the ingredients in a compounded polymer is often a difficult task. A complex mixture of compounds is involved, ranging from polymers to organic additives of various types. Indirect methods of separating the ingredients and identifying them have been developed. This study seeks to investigate additives by direct analysis of the substance. The organic additives are often at concentrations of 0.1-10%. The subject of this paper is laser desorption (LD) of polymer additives and laser desorption of neutrals of polymer additives with postionization (LD/postionization) by time-of-flight mass spectrometry. Although laser desorption alone has achieved some success in analysis of organic compounds, the desorption laser also acts as the ionization laser, limiting the ion yield, and thus the sensitivity. The main advantage of postionization techniques results from the separation of the desorption and ionization processes. We demonstrate the practical utility of this combination in the analysis of polymer additives.

EXPERIMENTAL

The instrument used here is a time-of-flight mass spectrometer constructed in-house. The mass spectrometer is established by a sample surface, an acceleration field, and field-free region with an ion detector at the end. The overall flight path length is 120 cm with acceleration distances of 4 mm for each of the two acceleration regions. Ions are produced at threshold irradiances to minimize fragmentation of the desorbing molecules. The ions thus formed are accelerated to 10 keV and detected using a dual-channelplate detector with a modest post-acceleration potential. Data are recorded in a LeCroy 8828 transient recorder with a maximum time resolution of 5 ns or by single-pulse counting in a LeCroy 4208 time-to-digital converter with a time resolution of 1 ns. Further processing of the data is accomplished in a PC-based software system. The typical operating vacuum is 5 x 10⁻⁹ torr. The lasers used are a 120-ps 266 nm Nd:YAG for desorption and a 10-ns 308 nm XeCl Excimer for postionization.

RESULTS

The samples used in this study were provided by James C. Tou (Dow Chemical Co.) (additives: tinuvin-234 and decabromo DPO) and Robert Lattimer (B. F. Goodrich) (additive: polychloroprene rubber vulcanizates A, B, C). The pure samples of the additives were dissolved in methylene chloride and placed on the stainless steel sample probe tip. The direct analysis of the rubber vulcanizates was accomplished by cutting small pieces of the rubber with a razor blade and retaining the piece on the sample probe tip by a copper disc with a quarter inch hole in it. The copper disc also serves to define an acceleration field for the sample which is non-conducting.

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The 39th ASMS Conference on Mass Spectrometry and Allied Topics

The LD/TOF mass spectra of several pure additive compounds were recorded. The mass spectrum of the negative ion of tinuvin-234 (a UV stabilizer) is shown in Fig. 1. An intense negative molecular ion signal is present with very little fragmentation. The positive ion mass spectrum also shows an intense molecular ion with weak fragment ions corresponding to loss of the hydroxyl group and phenyl groups. The flame retardant decabromodiphenyloxide shows an intense ion series. A positive and negative molecular ion is present. Masses above the molecular ion region are indicative of higher molecular weight brominated species.

The top panel in Fig. 2 is the direct analysis LD/TOF mass spectrum of the rubber vulcanizate B. Ions corresponding to the antiozonant component N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (HPPD) at m/z 268 are barely present among a large background of ions from other rubber additives and compounds. The much cleaner mass spectrum shown in the lower panel of Fig. 2 results from the reduction of the desorption laser power and addition of the postionization laser. The desorption laser power was reduced so that no prompt ions are observed. The laser irradiance was not recorded. The resulting spectrum show a single peak in the mass spectrum at m/z 268 (M++) characteristic of HPPD. At higher ionization irradiances the characteristic fragment ion (M-C4H9)+ is observed.

CONCLUSIONS

LD/TOF using threshold desorption of ions can be used as an analytical tool in the study of additives in polymers. Laser postionization of desorbed neutral molecules allows selectivity in detection of additives by direct analysis. Since different additives have different UV absorbances, careful selection of the ionization laser wavelength is expected to enhance the selectivity and sensitivity for additives. Future studies utilizing separation of the desorption and ionization processes will be used to record velocity distributions of the desorbed neutral molecules.

Fig. 1 The LD/TOF negative ion mass spectrum of the UV stabilizer tinuvin-234 (a benzotriazole).

Fig. 2 The direct analysis of the rubber vulcanizate B by LD/TOF (upper panel). The postionized neutral molecules of HPPD at m/z 268 is shown in the lower panel.
Matrix-assisted UV Laser Desorption of Proteins Using a 600 Picosecond Nitrogen Laser

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A compact laser desorption time-of-flight instrument utilizing an inexpensive, short-pulsed, and low-powered nitrogen laser has been constructed for analysis of proteins and their glycoconjugates. In addition to the simplicity of laser operation, the instrument features a multiple sample inlet system and minimal maintenance requirements thus enhancing its utility. A variety of protein and glycoprotein samples have been analyzed to demonstrate sensitivity, high mass range, resolution and mixture analysis.

The $N_2$ laser produces a 1.2 millijoule pulse in 600 picoseconds. Power utilization varies between 25 and 40% of the total pulse energy as determined by a Molelectron joulemeter. Pulse power reproducibility is better than +/- 4%. The TOF analyzer incorporates an orthogonal multi-sample inlet system, up to 30KV extraction, Einsel focussing, one meter flight tube and an electron multiplier detector. Extraction lenses have been optimized using SIMION computer simulations. Data collection is performed using a LeCroy 9400A or 9450 digital oscilloscope linked to a 386 PC. Pulse triggering is accomplished using a fast (<1 ns) photodiode. [Figure 1]
Femtomolar sensitivity, single shot, has been demonstrated. [Figure 2] Signal integration/averaging significantly enhances signal/noise ratios. [Figure 3] Mass range is in excess of 100 kDa. [Figure 4] Mass accuracy is greater than 0.1%. Picomolar protein mixtures can be analyzed. [Figure 5] Samples can be desorbed directly from nitrocellulose. [Figure 6] Observed peak widths less than mass 2000 parallel isotopic distribution widths for those compounds. [Figure 7] Since the transient recorder approaches 1 channel/amu while sampling a detector whose rise time is one-half the channel width, unit resolution at masses greater than 500 will be masked. Currently, the resolution is thus limited by the by the speed of the transient recorder and the detector. Faster devices will be required to determine the actual resolution limit using high-voltage extraction with short-pulse desorption.
MATRIX-ASSISTED LASER DESORPTION AND IONIZATION OF OLIGOSACCHARIDES

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We report here the application of matrix-assisted laser desorption mass spectrometry to the molecular mass determination of glycoprotein-derived complex and high mannose oligosaccharides obtained from the Dionex Corporation, (Sunnyvale, CA). Because underivatized oligosaccharides did not yield useful mass spectra, all the measurements reported here were performed on peracetylated compounds.

The linear time-of-flight laser desorption mass spectrometer was built at the Rockefeller University. The desorption and ionization are effected by frequency tripled radiation (354 nm) from a pulsed (10 nsec) Nd-YAG laser. All the oligosaccharides were peracetylated using a 2:1 (v:v) mixture of trifluoroacetic anhydride and glacial acetic acid. The matrices used were sinapic acid (3,5 dimethoxy-4-hydroxy cinnamic acid), ferulic acid (3-methoxy-4-hydroxy cinnamic acid), and caffeic acid (3,4 dihydroxy cinnamic acid). Two pmol of peracetylated oligosaccharide and 0.5 pmol of calibrant (usually neurotensin and bovine insulin) in 25 nmol of matrix in 0.5 ul of 1:2 acetonitrile and 0.1% trifluoroacetic acid was loaded onto the probe tip for mass spectral sampling.

Figure 1a shows the negative ion mass spectrum obtained for a disialylated bi-antennary oligosaccharide. The spectrum exhibits an intense (M-H)⁻ ion peak produced by deprotonation of the peracetylated molecule as well as two prominent peaks that are 42 u lower and 54 u higher than the (M-H)⁻. The former arises from the underacetylated oligosaccharide while the latter arises from the oligosaccharide containing one trifluoroacetyl group instead of an acetyl group. Analogous side reaction products have previously been observed on peracetylation of carbohydrates. The negative ion mass spectrum (Figure 1b) of a trisialylated tri-antennary oligosaccharide shows an (M-H)⁻ peak from the completely acetylated oligosaccharide and intense peaks that are 42 u apart $\text{(M-n(42)-H)}^-$ arising from different degrees of underacetylation. Thus negative ion formation occurs primarily by deprotonation in the case of complex carbohydrates containing acidic sugar residues.

The negative ion mass spectrum of the simple neutral sugar, maltoheptaose consists of a peak corresponding to (M+36)⁺ and another that is 42 u lower in mass. We assign the former as an (M+Cl)⁺ and the latter to its underacetylated analogue. Figure 1c shows the negative ion mass spectrum of a neutral, high mannose oligosaccharide. The peaks observed are (M+36)⁺, i.e. (M+Cl)⁺, and its analogues corresponding to underacetylated carbohydrate molecules $(M-n(42)+36)^+$ where $n = 1,2,3$, etc.). A peak arising from chloride ion attachment to the carbohydrate molecule [in which an acetyl group is replaced by a trifluoroacetyl group $(M-CH_2CO+CF_3CO+36)^+$ or $(M+54+36)^+$] is also observed. Similarly the mass spectrum of an asialo tri-antennary complex carbohydrate (Figure 1d) shows the (M+Cl)⁺ ion and it's underacetylated analogues. Thus, negative ion formation appeared to occur primarily by chloride ion attachment in the case of neutral carbohydrates. Truncated forms of the carbohydrate with loss of 1,2 or 3 terminal sugar residues were observed to a limited extent for all the carbohydrates studied. These are likely to arise from truncated impurity oligosaccharides in the samples studied.

Negative ion spectra of large complex carbohydrates are in general more intense, better resolved and easier to interpret than the corresponding positive ion spectra. The mass spectral sensitivity of these carbohydrates is extremely high (2 pmol) in the negative ion mode. This high sensitivity of matrix assisted LDMS provides a means for molecular mass measurement and structural studies of large biological carbohydrates that are available in extremely small amounts.
Figure 1
MATRIX-ASSISTED LASER DESORPTION OF HIGH MOLECULAR WEIGHT IONS USING TIME-OF-FLIGHT MASS SPECTROMETERS WITH AND WITHOUT REFLECTOR

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Matrix-Assisted Laser Desorption (MALD) of ions is a desorption/ionization method of rising interest, which allows to determine the molecular weight of biological compounds with very high mass (up to 150,000 u and more).

As it is quite new, many important questions are still open. Our special interest are effects which limit the resolution and the accuracy of mass determination in a time-of-flight mass spectrometer. In the literature, Beavis and Chait [1] obtained better resolution with a linear TOF as Hillenkamp and Karas [2] with a reflector TOF. The basic difference between the two experiments was that Beavis and Chait used very high ion energies (20 keV and more), whereas Hillenkamp and Karas used only 3 keV. Therefore we studied all combinations of low/high ion energy, linear/reflective TOF, postacceleration/no post acceleration for detection.

The best results were obtained with high ion energies and without postacceleration in the linear and reflector TOF. The highest sensitivity was obtained in the linear TOF and the highest resolution in the reflector TOF. Fig. 1 and Fig. 2 show the results for cytochrome C in a linear and reflecting TOF. In both cases, a sharp peak is observed which comes out of a broader one. This sharp peak is much easier to obtain, when high ion energies are used. In several cases, the sharp peak could only be obtained when high ion energies were applied. If this peak is absent, the resolution is obviously lower. Still the question arises why "only" a resolution of 1,200 for mass 12,000 can be obtained with a mass analyzer which is able to perform a resolution of 5,000 for mass 2,000 with another ion source [3]. The answer is that the isotopic distribution of the high mass molecules hinder the measurement of the resolution as long as the isotopic pattern is not resolved. For example, the FWHH of the isotopic pattern of mass 12,000 is about 8 masses. Therefore the maximum resolution theoretically obtainable with unresolved isotopic pattern is 1,500.

References:
Fig. 1: Shape of the molecular ion peak of Cytochrome C, recorded with 20 keV ion energy in a linear TOF with 140 cm flightpath. Matrix sinapinic acid, desorption wavelength 337 nm.

Fig. 2: Shape of the molecular ion peak of Cytochrome C, recorded with 16 keV ion energy in a reflector TOF with 165 cm flightpath. Matrix DHB, 337 nm.
APPLICATIONS OF MATRIX-ASSISTED LASER DESORPTION
MASS SPECTROMETRY TO BIOLOGICAL PROBLEMS

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Matrix-assisted laser desorption time-of-flight mass spectrometry, as first introduced
by Hillenkamp and Karas, and as later modified by Chait and Beavis, offers many new and
exciting possibilities for the structural determination of biologically important compounds.
We have begun to utilize this technique as part of our overall approach to the study of
proteins, glycoproteins, glycolipids, oligosaccharides, and oligonucleotides and related
compounds. Our applications, including the projects described below as specific examples,
exploit its advantages of high mass range, high sensitivity and tolerance for the presence
of impurities.

Experimental Laser desorption mass spectra were acquired with a VESTEC 2000
instrument, using a Lumonics Nd:YAG laser at 355 nm, 9 ns pulses, 5 Hz. Accelerating
voltage 20-30 kV. Details of the instrument and its operation appear in Abstract MP111.

Core amyloid protein isolated at autopsy from persons suffering from Alzheimer's disease
who succumbed to it (Collaborator: D. L. Miller, NY State Institute for Basic Research,
Staten Island).

The analysis of components of interest after minimal workup from biological materials
is exemplified by the investigation of amyloid proteins present in neuritic plaque cores
obtained from autopsy samples of the brains of patients who suffered from Alzheimer's
disease. The protein is difficult to isolate and purify, with major losses of material
occurring during the purification step. Previous work during this collaboration has shown
that the protein has a frayed N-terminus. In order to complete the characterization of
the protein, it was necessary to determine whether the C-terminus was also frayed. Both
the highly purified proteins and the crude plaque have been analyzed by maLD-TOF. The
results (Fig. 1) demonstrated that the C-terminus is not frayed, although the N-terminal is
extensively frayed. This spectrum of crude material contains multiple peaks separated by
28 u intervals for each peptide, because of partial formylation of the native material during
the extraction.

![Fig. 1. Matrix-assisted laser desorption mass spectrum of a crude (70% formic acid)
extract of amyloid plaque from the brains of deceased Alzheimer's disease patients.
Intact core amyloid peptide has 42 residues, but it is extensively and variously truncated
in the disease state. Sinapinic acid matrix.](image-url)
Analysis of the five glycosylation sites of human α-acid glycoprotein (orosomucoid glycoprotein) (Collaborators: M.J. Treuheit and H. B. Halseall, Dept. of Chemistry, Univ. of Cincinnati).

Orosomucoid (OMD) is a plasma glycoprotein (MW ca. 45 kDa) whose physiological function remains unknown, but it is classified as one of the positive acute phase reactants because its plasma level rises as much as fourfold in some disease states. It is one of the most heavily glycosylated serum proteins, and has glycan heterogeneity at each of the glycosylation sites. Variation in the extent of processing creates discrete glycoforms that may lead to functional diversity. Chromatographic methods for resolution of the various glycoforms have been developed. Determination of the molecular weights of glycopeptides (2-6 kDa) obtained by enzymatic digestion of OMD has been accomplished by FABMS and MALD-TOF/MS analysis of seventeen HPLC fractions. The latter MS approach required much less sample, was more successful in detecting the presence of minor glycopeptides, and more tolerant of impurities. A typical spectrum obtained for 35 pmole of glycopeptide is shown in Fig. 2. FABMS spectra, on the other hand, did contain some sequence information. Several glycoforms not detected by HPLC alone were identified by mass spectrometry.

Determination of the points of truncation of modified His-tRNA synthetase (Collaborators: P. R. Schimmel et al., Dept. of Biology, MIT).

Matrix-assisted LD-TOF analysis has shown that a variant of His-tRNA synthetase (M, 47.0 kDa, 424 amino acids) which was known to have a truncated N-terminus, has also lost a part of the C-terminus. The modified form appears to have adenylate synthesis but not aminoacylation activity and is apparently produced by a cleavage of the full-length His-tRNA synthetase by a cellular protease. While detection of the N-terminal modification may be fairly easily accomplished by Edman gas-phase sequencing, unambiguous detection of the C-terminal truncation is much more difficult, if not impossible. The molecular weight of the modified enzyme was shown by mALD-TOF/MS to be 39.6 kDa, indicating that the protein lacks not only the first 63 amino acids, but also the last 14 amino acids. An additional form which contains residues 1-410 (MW 45.6 kDa) was also detected.

Acknowledgement. The MIT Mass Spectrometry Facility is supported by Grant No. RR00317, from the NIH Center for Research Resources.
HIGH CURRENT ION SOURCE EXTRACTOR FOR QUADRUPOLE MASS SPECTROMETERS

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Introduction:
High current ion sources, such as inductively-coupled plasmas, microwave plasmas, and glow discharges used for elemental ionization are permitting new approaches to analytical mass spectrometry. As such, most instrument designs to date use only low voltage (0-200 V dc) extractors and transport lenses. The extraction of ions from high current ion sources has been extensively studied and used in isotope separator spectrometers. The design objectives for these systems are maximum ion extraction and transport efficiency, and minimization of space charge induced effects. With these goals in mind, a simple acceleration-deceleration ion-extraction system has been built using some of the principles of high current ion extraction. In this paper we present the results obtained with this lens design using a microwave ion source and a quadrupole mass spectrometer.

Basic Theory of Ion Extraction:
Ion extraction from an ion source is achieved by the application of a voltage between the ion source and the extraction electrode. The applied field strength, shape of the emitting surface, and space-charge density of the beam affect the trajectories which ions will undertake. The beam quality or phase space depends on the absolute current and the current density. The maximum space charge limited current density which can be extracted from any ion source is described by the Child-Langmuir equation and is proportional to $V^{3/2}/d^2$, where $V$ is the acceleration voltage, and $d$ is the gap width between the extractor and the ion source. Thus the larger the extraction voltage and smaller the gap width, the greater the ion density which can be effectively extracted. Unfortunately quadrupole mass spectrometers do not tolerate ions with kinetic energies much greater than 50 eV without large losses in mass resolution. Therefore, the ion beam must be decelerated just before entering the quadrupole mass spectrometer.

The Ion Source:
The ion source and mass spectrometer arrangement is shown in Figure 1. Although the ultimate use of this mass spectrometer is for a variety of other ion sources, we chose to test it with a low pressure microwave ion source utilizing a $\lambda/4$ wave Swenson type cavity in a 0.5" o.d. fired alumina tube. This type of ion source can easily produce current densities in the order of 25 mA/cm$^2$ at 5-200 mTorr gas pressures. In our Division this ion source is regularly used to provide 1-10 nA ion beams of various elemental species for isotope separation. Argon gas is used as the plasma support gas, and a calibrated xenon gas leak of $1.2\times10^{-6}$ atm cc/s was used for efficiency tests. The ion source extraction orifice has a 0.0135" diameter. With this orifice, and a 330 L/s turbo pump, the main mass spectrometer pressure was maintained at 5x10$^{-5}$ to 5x10$^{-7}$ Torr pressure, depending on the ion source pressure.

The Accel/Decel Lens and Mass Spectrometer Arrangement:
The extractor lens, placed just behind the ion source orifice, gives a maximum ion signal at ~8000 V/in extraction field. The complete lens is comprised of two more lenses just in front of the quadrupole mass spectrometer. The ions are decelerated to 50 eV as they travel through the quadrupole. This value results from a compromise between resolution and signal intensity. Although the mass spectrometer includes an analog electron multiplier, this was seldom used since the ion signals observed were high enough that recording with the built in faraday cup was sufficient for determination of system performance.
System Performance:
Figure 2 shows the first background mass spectrum, and RF only spectrum recorded with this system. The main signal is due to the argon gas at mass 40 (Ar⁺), with minor peaks at mass 41 (ArH⁺), 80 (Ar₂⁺), 28 (N₂⁺), and some smaller peaks in the mass 12-19 region due to water, N⁺, and C⁺. Although the baseline looks very clean upon expansion or recording with the analog multiplier a "picket fence" of peaks is uncovered which attests to the high efficiency of the microwave ion source in ionizing any contaminants present in the source or plasma gas. The RF only spectrum shows 4X more signal, indicating that 75% of the signal is lost in the quadrupole when operating at unit mass resolution. The maximum Ar⁺ signal we have been able to observe with this system is 250 nA at unit mass resolution.

Figure 3 shows a spectrum of the xenon signal obtained with the calibrated leak. A total xenon signal of 60 nA could be recorded on a regular basis with this system. Therefore the detection efficiency of the system is:

\[
\text{Xe leak} = 1.2 \times 10^{-6} \text{ atm cc/s} = 3.0 \times 10^{13} \text{ atoms/s} \\
60 \text{ nA Xe} = 3.6 \times 10^{11} \text{ ions/s} \\
\text{Detection Efficiency} = 1.2\%
\]

Since at least 75% of the ions are lost in the quadrupole, the minimum ionization and transport efficiency of this system is 4.8%. Thus, the accel-decel lens design for this system is very efficient in the transport of ions from the high current microwave ion source to the quadrupole mass spectrometer.
COMPARISON OF PULSED DC AND PULSED RF GLOW DISCHARGE MASS SPECTROMETRY

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INTRODUCTION

Glow discharge mass spectrometry (GDMS) is regarded as a useful analytical technique for trace elemental analysis of solid materials. Most of the popularity of GDMS has been with the use of direct current (DC) discharges operating at equilibrium. There are, however, alternative glow discharge techniques that offer advantages over the traditional DC source.

Two alternative glow discharge methods are pulsed DC and radio frequency (RF) discharges. Pulsed DC discharges periodically turn-off the applied voltage at a rate of 50 - 100 Hz with 50% duty cycles. They offer higher sputter yields and ion signals than their DC counterpart and produce ion signals useful for fundamental studies and analytical applications (1). Radio frequency discharges rely on the difference in mobilities between electrons and ions in an oscillating (low MHz) electric field to create a DC bias voltage on the sample surface that controls sputtering. Nonconducting materials as well as conducting samples can be analyzed directly with RF discharges.

Reported here is a new ion source whereby an RF discharge is pulsed ON-OFF by periodically modulating the applied voltage. The pulsed RF discharge is evaluated by comparing its ion signals with those obtained with a pulsed DC discharge operating at the same voltage.

RESULTS AND DISCUSSION

Figure 1 illustrates the ion signal profiles for both pulsed DC and pulsed RF discharges. Sputtered species ($^{57}$Fe$^+$ in Figure 1a) show the characteristic afterpeak observed in an argon discharge when the applied voltage is terminated; this is the result of Penning ionization becoming dominant in the post-pulse region as electrons rapidly thermalize. Likewise, contaminant gas species (Figure 1b, $^{18}$(H$_2$O)$^+$) in both discharges exhibit prepeaks as electrons pass through their
optimal collisional cross section as the applied voltage increases. While the signal profiles for pulsed DC and pulsed RF are very similar, there are differences between the two discharges. At the beginning of the pulse period, sputtered ion signals in a pulsed DC discharge typically appear around 0.8 msec into the pulse cycle after the high voltage is applied to the sample. In a pulsed RF discharge, the same ion signals appear approximately 0.4 msec earlier in the pulse period. This is the result of differences in how the RF and DC discharges are pulsed. In a DC discharge, it is the power supply that responds to a square wave controlling the pulse cycle. RF discharges respond to the controlling wave by changing the amplitude of the RF voltage, but the discharge is actually pulsed because ion bombardment neutralizes the DC bias. Once ions are no longer present, DC bias neutralization stops, leaving a residual voltage between 150 - 200 V on the sample surface. At the beginning of the next pulse cycle, the DC pulse must start at zero voltage, whereas the RF pulse begins at a voltage just below the threshold to initiate the negative glow.

There are also differences in the ion signals when the pulse discharge stabilizes. Figure 2 is a mass spectrum of the molybdenum region of a low alloy steel (SRM 1264). In the figure, solid peaks are from the pulsed DC spectrum while the outlined peaks are a pulsed RF mass spectrum. The DC voltage and RF bias voltage were matched at -1000 V. The pulsed RF discharge consistently yields higher ion signals than the pulsed DC discharge.

When the discharge is terminated, the afterpeaks in the two pulsed discharges are also slightly different. Figure 3 is a trace of the afterpeak region for $^{57}$Fe*. The plateau values of the two discharges are matched to draw attention to differences in the shape of the afterpeak. Pulsed RF discharges consistently produce afterpeaks that are broader than pulsed DC afterpeaks. Because the afterpeak region is dominated by Penning ionization, differences in the afterpeak profiles suggest that the population of discharge gas metastable atoms are not equal in the two discharges. To examine this possibility, atomic absorption was used to monitor the population of metastable atoms in the discharge immediately in front of the exit orifice. While absolute concentrations cannot be determined, the relative metastable population between the two discharge can be ascertained. These studies indicate that the argon metastable population in the pulsed RF discharge is 8 - 12% higher than in the pulsed DC system. This would explain why the afterpeaks are broader in the RF discharge. The higher argon metastable population may also contribute to the enhanced ion signals observed in the plateau region of the pulsed RF discharge.

**INTRODUCTION**

In recent years, glow discharge mass spectrometry (GDMS) has developed into a suitable technique for the analysis of solid samples. The applicability of GDMS to solution samples is limited, however, due to the low operating pressures of the plasma. Perturbing effects will be encountered in both the plasma and the high vacuum system if a large quantity of solvent is allowed to enter the system. In an effort to explore possible methods of solution analysis, an electrothermal vaporization (ETV) source is currently under investigation in this laboratory. The ETV device chosen for these studies takes the form of a coiled filament (tungsten or rhenium). The method used in these experiments is evaporation of the solvent from a solution sample (using the filament at a low current) resulting in a solution residue on the filament surface. The residue is then electrothermally vaporized when the filament is heated to a temperature above the vaporization point of the analyte, thus producing atoms that may be analyzed in the negative glow region of the discharge. In these analyses, the heating of the filament serves as the primary atomization step, while the glow discharge provides the only apparent ionization mechanisms. This presentation focuses on the fundamental characteristics of the system to find the optimum parameters to be used for further studies.

**EXPERIMENTAL**

Figure 1 shows the ion source configuration used in these experiments. The filament coil, attached to the end of a 1/2" stainless steel direct insertion probe, serves both as the cathode of the glow discharge as well as completing a current loop by attachment to a constant current power supply. The aqueous solutions (prepared by mixing the appropriate metal nitrate with distilled water) are analyzed by placing a 5-50 µl sample onto the coil with a micropipet. The solvent is then evaporated external to the mass spectrometer ion source at a relatively low current (the current averages between 1.5 and 2 amps and is filament dependent). The probe is then inserted into the mass spectrometer for subsequent analysis. The number of turns used for the electrothermal filament is dependent on the filament material. This value is determined by the length of wire required to produce a white hot glow using about 4 amps current. The ionization and detection are provided by the GDMS system.

**RESULTS AND DISCUSSION**

Filament Current Effects: Before proceeding with solution analysis, the initial investigations on this system were to observe the effects that the filament current has on the glow discharge system. Two experiments were conducted: one on a constant voltage discharge and one with a constant current discharge. The observed trends with the constant voltage discharge were the same for all species under investigation (three gaseous species and one sputtered species). Upon initiation of the filament current (from 0-1.5 amps) the signal begins a slow decrease and then levels off for a period (1.5-2.5 amps), but from 2.5 amps to the maximum used (4 amps) there is a sharp increase in the ion...
signals. The underlying reason for this trend is discovered when monitoring the glow discharge current which follows a similar trend. These effects are most likely due to the changing electron population in the plasma. When using a constant current discharge, the gaseous species show a sharp increase with increase of the filament current that is probably due to source heating and degassing. The sputtered species, however, show a slight increase until the filament glows incandescent and then decreases. This is probably due to the shift in optimum cathode to exit orifice distance upon influx of a large amount of electrons. This is further explained in the distance studies below.

Pressure Studies: An important parameter to be considered for glow discharge operation is the effects of the source pressure. As the pressure is increased from about 0.5 Torr, the ion signal will increase to a maximum and then decrease. This is due to the changing amount of argon metastable atoms in the discharge which controls the amount of Penning ionization. Subsequent experiments were performed at the optimum pressure because of the higher ion signal and also because any change in pressure results in a minimal ion signal change. The studies revealed that the optimum pressure for both normal discharge analysis and ETV analysis occurred at 1.3 Torr argon.

Cathode-Exit Orifice Distance Studies: Another important parameter to investigate is the change in ion signal with variation of the sampling distance. Since all detected ions come from a small zone very near the exit orifice, the distance for maximum signal should be found. These studies produced an interesting difference. Figure 2A shows the distance effects for a normal sputtered species. The maximum ion signal is obtained at about 7 mm, which is typical for GDMS studies. The unexpected finding is seen in Figure 2B, which shows that when the filament is running at 4 amperes (normal operating conditions for solution analysis) the ion signals from both the cathode material and the solution residue show a maximum at about 3-4 mm. This could be a result of the differing amount of electrons that will affect the argon metastable population and the plasma conditions. Future work with atomic absorption should clarify this.

CONCLUSIONS

Electrothermal vaporization has been shown to provide quick quantitative analysis when incorporated with a GD ion source. The ETV filament provides a nearly complete atomic population from the solution residue that is subsequently ionized and detected in the GDMS system. The optimum conditions for this system are similar to those of a normal GDMS system. The pressure for maximum ion signal was found to be about 1.3 Torr argon which is in the range of normal operating parameters, but this will change slightly from system to system. The parameter diverging most from a normal GDMS system is that of the cathode-orifice distance which decreases when the filament is running at a white hot temperature. The normal distance of about 7 mm decreases to about 3 mm. This is probably due to changes in the plasma and ionization mechanisms with the increase of the electron population.
INTRODUCTION: The glow discharge plasma, formed about cathodes containing metal oxides, is more complex in its chemical composition than that with bulk metals. The complexity arises from high concentrations of gaseous impurities brought into the plasma by the compressed samples. The effect of these gaseous species on gas phase chemistry of the oxide has been studied with direct current GDMS. It is realized that the presence of these gaseous contaminants on the cathode surface may also influence the oxide’s plasma processes, especially its sputtering mechanism. However, the degree of gas contamination on the cathode surface is difficult to monitor in a dc discharge, because the cathode surface remains at a “chemical steady state” after the initial sputter-cleaning step. In order to investigate the effect of cathode surface contamination on oxide signals, pulse GDMS was used to create a series of sample surfaces with varying yet controllable amounts of gaseous impurities.

EXPERIMENTAL: A pulse generator with variable pulse period in the range of 1-3500 milliseconds was used to drive a high voltage dc power supply. The discharge was maintained in 1.0 Torr Ar, at constant 1200 Volts during the pulse “on” time. The analyte, lanthanum oxide (La₂O₃), was mixed with two metal matrices, Ta and Ag, to form disk cathodes. All data were collected through a 1 ms data gate placed in the pulse “on” time region.

RESULTS AND DISCUSSION:

Pulse Schematics: Throughout the experiments discussed in this section, the pulse “on” time was kept at a constant 5 ms while the pulse “off” time was varied between 10 and 200 ms. As shown in Figure 1, following the pulse “on-off” cycle, the cathode undergoes repetitively the processes of sputtering/sample heating (during the “on” time) and cooling/surface deposition (during the “off” time). For completing the same number of pulse cycles, if the length of the pulse “off” time is increased, then the cathode will have longer time to dissipate heat and to accumulate surface deposition, resulting in reduced cathode temperature and increased amounts of gaseous impurities on the sample surface. By the same token, reducing the length of the pulse “off” time results in less surface accumulation of gases as well more pronounced cathode heating.

Effect of Pulse Period Change on Water Signal: As the pulse period was changed, the water signal (H₂O⁺) was monitored with four types of cathodes: bulk Cu pin, pressed Cu disk, pressed Ta/La₂O₃ disk, and Ag/La₂O₃ disk (Figure 2). The water signals show two different changing trends: one increases with increasing “off” time (Cu pin and Ta disk); the other decreases with “off” time (Pressed Cu and Ag disks). In the cases of Cu and Ag disks, water molecules trapped within the compressed electrodes are released from the sample into the discharge as the electrode heating is intensified in the low period region. For the Cu pin, with no diffusion from within the electrode, the water signal follows the surface accumulation pattern with the changing period. The Ta disk, although also compress-formed, does not
appear to release a large amount of water upon heating. This may be because heating activates Ta's gettering function, which dissolves water molecules enveloped inside the electrode into the metallic inside of the Ta granules, preventing them from escaping into the gas phase.

**Figure 2**

Effect of Surface Water on La⁺/LaO⁺ Ratio: The probable water accumulation on the Ta cathode surface has a direct influence on the La⁺/LaO⁺ ratio, as illustrated in Figure 3A. As the water signal increases with lengthened "off" time, the La⁺/LaO⁺ ratio gradually decreases. This is probably because the enhanced water deposition creates an "oxidizing" layer on the cathode surface, resulting in reduced sputtering ratio of the La/LaO neutrals. In order to confirm further that in the case of Ta matrix, the La⁺/LaO⁺ ratio is affected by water molecules deposited on the cathode surface from the gas phase, but not water vaporized from within the electrode, about 2% O labeled water was bled into the discharge gas to carry out the same pulse experiments. H₂¹⁸O⁺ signal and La⁺/La¹⁸O⁺ ratio were monitored (Figure 3A), showing similar trends as those O-containing species (Figure 3A). Since O exists only in the discharge gas phase with no history in the sample, then the strong similarities in Figures 3a & 3b tend to suggest a similar behavior between O and O waters in terms of affecting the oxide, i.e., through surface deposition during the pulse "off" time.

**Figure 3**

CONCLUSIONS: Pulse discharges were used to monitor the degree of gaseous deposition on the cathode surface, and to study the effects of such deposition on the oxide's discharge processes. Results show that water layers on the cathode surface may cause reduced elemental signal of the oxide. Studies also suggest that gettering action of Ta inside the heated electrode is possible.
A VERSATILE EXTERNAL SAMPLE MOUNT GEOMETRY FOR BULK AND THIN FILM ANALYSIS BY RADIO FREQUENCY GLOW DISCHARGE MASS SPECTROMETRY

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Glow discharge mass spectrometry (GDMS) has become a well accepted technique for the bulk solids elemental analysis of metals and semiconductors. In this laboratory we have addressed that class of materials that do not conduct electricity by developing a family of glow discharge devices which are powered at high frequencies (13.56 MHz), thus allowing the direct analysis of the full range of bulk materials. These radio frequency (rf) powered glow discharge devices have been applied for the sputter-atomization of such materials as nuclear defense waste glass and machinable glass ceramics (MACOR). Initial efforts in rf-GDMS involved a source design employing a direct insertion probe (DIP) for sample introduction. While the DIP allows convenient sample interchange through a vacuum interlock and the ability to vary the ion sampling position, the 0.5" probe diameter limits the analytical sample size to <0.25". Clearly, if the bulk analysis of glasses and ceramics is a primary benefit of the rf source, then limiting the sample size and thus imposing a machining requirement is a major drawback. To address this shortcoming and allow for convenient depth-resolved analyses, a simple external sample mount geometry has been constructed requiring only that the sample be nominally flat with a diameter of ~0.3". Other design considerations which must be addressed include the ability to, at least initially, vary the cathode-to-sampling cone distance and to permit rapid plasma equilibration for high sample throughput. The basis of this report is a description of this source design and illustration of its operating characteristics.

Experimental

Shown on the next page is a diagrammatic representation of the rf-GDMS external sample mount geometry source. The source is comprised of a three inch squared stainless steel cube which is mounted directly to the face plate of the double-quadrupole mass spectrometer (Extrel C-50) which serves as the anode of the discharge. The discharge is struck between the anode body and the cathode sample which is held against a Teflon o-ring by a brass torque bolt to form the vacuum seal. A bellows-type assembly allows for adjustment of the cathode-to-sampling cone distance by moving the sample and anode plate. The rf potential is applied directly to the back of the sample by a 1/8" copper rod attached to a type HN rf connector. A RF Plasma Products generator and impedance matching network (RF5 and AM 5) maintain the discharge at powers of 5-25 watts. Plasma ions enter the analyzer chamber through a 0.02" orifice in the sampling cone. The sampling cone can be biased with an external potential to act as a repeller for enhanced ion throughput. The rf-GDMS source is operated with argon as the discharge gas at pressures of 0.1-0.35 torr. Mass spectra are output to an X-Y plotter.
Results and Discussion

The new external sample mount geometry source has been evaluated on the basis of the plasma stabilization times, dependence of ion signal on sampling position and discharge conditions, and its ability to sputter-atomize both nonconductors and thin film systems. In order for GDMS to be acceptable for production applications, the sample analysis times must be appreciably shorter than the 30 minutes required for the current pin-type d.c. sources. Stabilization times have been found with the source under study here to be less than one minute with the plasma remaining stable to on the order of -3% RSD for periods of over one-half hour. At the outset of these studies, it was expected that the optimum sampling conditions for the external sample mount geometry would be appreciably different from those found for the DIP described at the 1990 ASMS Conference (paper #401) due to a much lower power density in the present case. Detailed studies indicate essentially no difference, with maximum analyte signal obtained for a sampling distance of 5mm and discharge conditions of 0.15 torr and 20 watts of rf power. Under these conditions, analyte ion beam currents on the order of a few hundred picoamps are obtained. Analysis of glass and thin film samples have been possible with the source though the current 1" inner diameter of the bellows assembly is still somewhat limiting in the types of samples that can be analyzed. Future developments will focus on the use of a single sampling position arrangement, as is used for emission spectroscopy in this laboratory, which will remove the diameter restriction currently experienced and also result in a much simpler construction.
Glow discharge mass spectrometry (GDMS) is proving itself to be a valuable tool for bulk elemental analysis of metals and semiconductors. In this laboratory, we have extended the applicability of the technique to nonconductors by use of radio frequency (13.56 MHz) powered devices. In the rf glow discharge plasma, the electrons in the negative glow region experience a rapidly alternating set of field potentials, different from d.c. plasmas wherein the negative glow consists of negligible, static, fields. This difference is manifested in pronounced differences in the discharge plasma potential from which the ions are extracted. In terms of performing quadrupole mass spectrometry, this presents problems in that the kinetic energies of the rf-produced ions will be higher than in the d.c. case. With the aid of quadrupole offset potentials, energy differences may not be appreciable so long as the width of the kinetic energy packet is narrow. Another reason for differences in the kinetic energies is the fact that the actual ionization mechanisms may be different. In our rf source electron impact seems to be the dominant pathway, while Penning ionization seems to dominate in d.c. plasmas. The studies described here address efforts to increase the ion transmission efficiency in quadrupole rf-GDMS and also to gain a more thorough understanding of the ionization environments in rf and d.c. plasma sources.

**Experimental**

The radio frequency glow discharge ion source and double-quadrupole mass spectrometer system are shown on the accompanying page. The external sample mount geometry source allows analysis of nominally flat, conductive or nonconductive samples of greater than \(-0.3\)" diameter. The source operates with argon as the discharge gas at pressures of 0.1-0.35 torr, and with rf powers of 5-25 watts. The double-quadrupole system was operated with the first quadrupole (Q1) in an rf-only mode without any collision gas present. Ion kinetic energy measurements were performed by use of the quadrupole retarding potential method. In particular, the d.c. offset voltage of the mass analyzing quadrupole (Q2) was varied with the corresponding effects on ion intensities noted.
Results and Discussion

Ion kinetic energy studies confirmed earlier Langmuir probe measurements performed in rf and d.c. plasmas in this laboratory. In all of the cases, the mean ion kinetic energy values were of the order of 16-18 eV, the precise range of the plasma potential measurements. Interestingly, the energy distributions for monoatomic ions were far more narrow than those for molecular ions such as dimers and argides. This may be explained by the fact that molecular species are formed by two-body associated ionization reactions rather than simple EI or Penning collisions. Alternatively, these species may be formed not in the plasma, but in the gas expansion beyond the sampling orifice. A suite of studies were performed to elucidate the role of extraction optics on the kinetic energy distributions. It was found that the optics systems had negligible effects on the energy distributions, but were important mostly on absolute sensitivity. It was interesting to note that, of all of the ion optical elements, the "skimmer cone" potential had the most dramatic effect on the relative intensities of the various ion species. Finally, studies of the role that plasma parameters play indicated substantial influence on the ion energies. These effects are most certainly related to changes in the plasma potentials.
An Appraisal of the Glow Discharge as an Elemental Ion Source for Quadrupole, Magnetic Sector, and FTICR Mass Spectrometers

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Techniques of Fourier transform ion cyclotron resonance (FTICR) mass spectrometry have been applied to a wide range of chemical problems, from ion photodissociation to comparisons of gas phase and solution reactivity to analyses of polymers and biopolymers. Until the development of external ion generation/injection, ion formation had been limited to sources that could operate under the constraint imposed by the low pressure analyzer. This precluded the use of fast-atom bombardment, supersonic expansion/photoionization, as well as several more conventional elemental ion sources, such as the inductively coupled plasma (ICP) and the glow discharge (GD). Recently, however, we have interfaced an abnormal glow discharge ion source to a Nicolet FTMS 1000 Fourier transform ion cyclotron resonance (FTICR) mass spectrometer, bringing the total number of different types of GD mass analyzers to four. While it can be argued that each GDMS instrument has a place in analytical mass spectrometry, we feel that the newest approach, GD-FTICR, offers several advantages over quadrupole and magnetic sector based instruments.

EXPERIMENTAL

The positive ions generated in the negative glow region of a coaxial cathode glow discharge were mass analyzed by a home-built FTICR mass spectrometer that employed a Nicolet FTMS-1000 electronics console to control a 4.50 cm x 2.54 cm x 2.54 cm ion analyzer cell. Figure 1 illustrates modifications necessary to interface the FTICR mass spectrometer to the glow discharge source. A series of four electrostatic lenses accelerated and focused ions sampled from a 1.0 mm diameter ion exit orifice. In the initial studies only one stage of differential pumping was employed; in this manner the analyzer pressure could be maintained at 2 x 10^-6 Torr (discharge pressure = 1.0 Torr). To obtain higher resolution mass spectra, the collision frequency in the ion analyzer cell was further reduced by placing a second 1.0 mm diameter orifice between the electrostatic lenses and the FTICR analyzer cell (Figure 1B). In this configuration the analyzer could be maintained at 9 X 10^-6 Torr, but with a concomitant loss in signal.

A typical pulse sequence involved dropping the analyzer cell trapping plates from +2V to ground for a period of 0.1 - 5.0 seconds to allow ions to be "injected" into the cell, and then raising the plates back to +2V for the detection phase. In typical experiments ions with frequencies from 10 kHz to 2.667 MHz were excited and detected with a bandwidth of 2.667 MHz. Fifty time domain signals of 16 K data points each were accumulated, and the average time domain signal was apodized by a modified Blackman-Harris window function and zero-filled once prior to Fourier transformation.

RESULTS AND DISCUSSION

Figure 2 shows the mass spectrum obtained when an NIST 1261a stainless steel pin is the sample cathode. The iron isotopes at m/z 54 and 56 are the major peaks along with interfering peaks at masses 18, 19, 28, and 29, resulting from H2O^+, H2O^+, N2^+, and N2H^+, respectively. Ar^+ and ArH^+ were ejected from the cell via a swept frequency ejection pulse and therefore don’t appear in the spectrum. Minor components of the sample at the 0.5 - 2% level are easily detected, and the isotopic ratios agree with known values to about ± 5%. Preliminary results indicate detection limits on the order of 25 ppm. Although the sensitivity is rather low when compared with other GDMS instruments, for which the detection limits are on the order of 1 ppb, improvements in our relatively simple ion injection scheme (for instance the addition of a Bessel Box energy analyzer and quadrupole ion guide) should lower the LOD by several orders of magnitude.

Moderate resolution of m/Δm = 10000 and high resolution of m/Δm = 40000 obtained in experiments to date indicate that the FTMS approach holds the potential for discrimination of isobaric...
interferences. Studies are also continuing to exploit the unique capability of ion trapping in providing other schemes for the elimination of glow discharge polyatomic interferences.

Figure 1. (A) A 2 Tesla Fourier transform ion cyclotron resonance mass spectrometer modified to accommodate a glow discharge ion source. (B) An expanded view of (A) showing the glow discharge ion source region in detail.

Figure 2. Low resolution GDFTICR mass spectrum of an NIST 1261a stainless steel cathode. 1.0 Torr argon; 1500 V d.c. discharge; $^{40}$Ar$^+$ and ArH$^+$ ejected.
Gas Phase Photochemistry of Anionic Metal Carbonyl Complexes.
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Introduction
This report involves two aspects of the gas phase photochemistry of anionic metal carbonyl complexes: (1) the photodissociation spectra and decomposition pathways of dinuclear metal carbonyl complexes \( \text{M}_2(\text{CO})_x^- \), \( \text{M} = \text{Mn, Fe, Co}, \ x = 4-9 \) and (2) the radiative relaxation of vibrationally excited \( \text{Cr(OC)}_6^- \) under collisionless conditions.

Experimental
All experiments were performed on an FT-ICR previously described in detail[1]. For the relaxation studies the metal complex precursor was introduced into the vacuum chamber via a pulsed valve. The relaxation study was commenced when the chamber pressure reached ~1.5 \times 10^{-4} \text{ torr}. All ions were generated with an ~10 usec 3 microamp beam of ~0.1 eV electrons.

The photodissociation studies of the dinuclear complexes were performed with a Coherent Ar* pumped cw dye laser. The output of the dye laser into the ICR cell can be gated on/off with a mechanical shutter which can open on the 2 msec timescale. The relaxation studies were performed with a Spectra Physics pulsed Nd:YAG operated in the long pulse mode where the temporal output consists of a train of ~200 10 nsec pulses produced in a 200 microsecond envelope.

Results
CO\(_3\)(CO\(_x\))\(_2\) and Fe\(_2\)(CO\(_x\))\(_2\), generated from dissociative electron capture by CO\(_2\)(CO\(_x\))\(_2\) and Fe\(_2\)(CO\(_x\))\(_2\), dissociate by simple loss of CO ligands in the 565 - 635 nm range. All the photoproducts also dissociate by CO loss except for the \( \text{M}_2(\text{CO})_4^- \) which dissociate exclusively by loss of M. The exclusive loss of a metal atom in the last step does not appear to be due to an energetic factor, because the lowest energy dissociation pathway for Fe\(_2\)(CO\(_x\))\(_2\) is formation of Fe(CO\(_3\))\(_2\) and Fe(CO). The unusual dissociation behavior may be due to photoexcitation to a repulsive state which eliminates a metal atom, or an activation barrier introduced by a change in spin between the photoexcited anion and the products.

The radiative relaxation of vibrationally excited \( \text{Cr(OC)}_6^- \) under collision free conditions was investigated using the two-pulse photodissociation method developed by Dunbar and coworkers[2]. The basic process is shown in scheme I. After initial photoexcitation with a 1064 nm photon \( \text{Cr(OC)}_6^- \) undergoes rapid interconversion to a vibrationally excited ground state. The relaxation of the vibrationally excited state was interrogated by irradiating the excited \( \text{Cr(OC)}_6^- \) with a second 1064 nm photon. By varying the time between the laser pulses and monitoring the amount of dissociation (loss of CO which requires two 1064 nm photons) it is possible to monitor the relaxation. The results are shown in Figure I.

Interestingly, the intermediate vibrationally excited \( \text{Cr(OC)}_6^- \) has considerably enhanced reactivity toward O\(_2\). The reactions and temperature dependence of the reaction has previously been studied[3]. This is the fourth reported observation of a photoinduced chemical reaction of a gas phase ion.

References
1) Buckner, S.W.; VanOrden, S.L.; Organometallics, (1990), 9, 1093.
Two-photon Dissociation Scheme:

\[ \text{Cr(CO)}_5^+ : \text{Rate Process Model} \]

![Diagram of Two-photon Dissociation Scheme](image-url)
TRACE METAL DETERMINATION BY GC-MS

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Introduction

Isotope dilution mass spectrometry can be used as a definitive analytical method for trace element determination providing reference values for concentrations in both normal and pathological conditions. We have used a general-purpose mass spectrometry system, preparing volatile-thermally stable metal chelates, for the determination of isotope ratios and metal concentrations. Historically, the lack of suitable chelating agents was the key limitation preventing the application of GC-MS to trace element analysis. The problems of poor accuracy and precision of the isotope ratio measurements resulted, in part, from cross-contamination or "memory effect" during sequential analyses of samples of widely varying isotopic compositions. We have evaluated 8 elements and 7 chelating agents.

Experimental

All the analyses were accomplished on a Finnigan MAT 8230 GC-MS system equipped with a 10m x 0.32 mm, non-polar fused silica capillary column, DB-1. Chelates were injected at an oven temperature of 100°C followed by a 25°C/min ramp to 300°C. The ion source was heated to a temperature of 200°C and the GC-MS interface was at 280°C. GC-MS data were acquired at 2 Hz yielding approximately 20 data points across the 10-s wide GC peak. A mass resolution of 1000 was used throughout.

Normal trace metals procedures to avoid contaminations were followed. The enriched isotope solutions used as internal standard for isotope dilution were calibrated by reverse-isotope dilution GC-MS using certified atomic absorption standards.

The 70 eV El mass spectra were recorded for all the chelates and the isotopic cluster of peaks with maximum intensity was used throughout the experiments. The isotope ratios were measured in the selected ion monitoring mode using voltage peak switching and the quantitation was achieved using the chromatographic peak areas.

Urine samples were digested with a wet oxidation procedure using concentrated HNO₃ and H₂O₂ for Ni, Cr, Co, Cu, Pb and Cd. For the determination of Pt and Se, wet digestion procedure with HNO₃ + H₃PO₄ (5:1, v/v) and H₂O₂ was employed. Blood and serum samples were first deproteinized using concentrated HNO₃ and the precipitated proteins were pelleted by centrifugation and the supernate digested as the urine samples. Lead (Pb) was determined in the whole blood; and Cu was determined in serum.

Precision and accuracy of the isotope ratio measurements was evaluated at 10 ng level by performing replicate analyses of samples on different days. Memory effect was investigated using synthetic mixtures of the natural primary standard solution and the enriched isotope solution in differing proportions but containing almost equal amounts of element. Isotope dilution GC-MS methodology was validated by determining trace elements in the National Institute of Standards and Technology (NIST) reference materials, SRM-2670 for urine and SRM-909 for serum.

Results and Discussion

Lithium bis(trifluoroethyl)dithiocarbamate, Li(FDEDTC), was successfully used for Ni, Cr, Co, Pt, Pb and Cd in the μg/L range. N,N'-ethylene bis(trifluoroacetyl)acetoneimine, H₂(enTFAJ, was used for Cu in serum and urine; while 4-trifluoromethyl-o-phenylenediamine was found to be the best chelating agent for Se. These chelating agents were synthesized in our laboratory.
No memory was observed for Ni, Cr, Co and Pt; however, a slight memory effect was observed with Pb and Cd which could be minimized by appropriate addition of the internal standard.

For the monoisotopic element Co, a novel approach using enriched isotope of Ni as an internal standard was used for its determination by GC-MS.

The values of trace elements determination by isotope dilution GC-MS were compared to the NIST certified/recommended values in the reference materials (Table 1). Good agreement between the expected and experimentally determined values proved the validity of isotope dilution GC-MS for trace elements determination at μg/L levels.

Conclusion

The results of the work todate indicate that isotope dilution GC-MS using chelating agents has great potential as an analytical method. However, it should be noted that each metal, chelate and GC-MS system should be carefully evaluated to establish the analytical limits.

Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Trace element</th>
<th>Matrix</th>
<th>Concentration (μg/L)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Determined</td>
<td>Expected</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Cr</td>
<td>Urine</td>
<td>92 ± 3 (n=11)</td>
<td>85 ± 6</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13 ± 1 (n=5)</td>
<td>13 ± ?</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ni</td>
<td>Urine</td>
<td>82 ± 3 (n=6)</td>
<td>70 ± ?</td>
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<tr>
<td>3</td>
<td>Co</td>
<td>Urine</td>
<td>104 ± 14 (n=6)</td>
<td>105 ± 6 (n=2)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.12 ± 0.24 (n=4)</td>
<td>1.37 ± 0.60 (n=4)</td>
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</tr>
<tr>
<td>4</td>
<td>Pt</td>
<td>Urine</td>
<td>125 ± 5 (n=6)</td>
<td>120 ± ?</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Pb</td>
<td>Urine</td>
<td>105 ± 4 (n=4)</td>
<td>109 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Whole Blood</td>
<td>288 ± 5</td>
<td>270 ± 110</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>806 ± 43</td>
<td>750 ± 250</td>
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<tr>
<td>6</td>
<td>Cd</td>
<td>Urine</td>
<td>94 ± 10 (n=6)</td>
<td>88 ± 3</td>
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<td></td>
<td>9.3 ± 0.9 (n=2)</td>
<td>8.4 ± 0.3</td>
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<tr>
<td>7</td>
<td>Cu</td>
<td>Urine</td>
<td>410 ± 30 (n=4)</td>
<td>370 ± 30</td>
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<td></td>
<td></td>
<td>Serum</td>
<td>1000 ± 30 (n=4)</td>
<td>1100 ± 100</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Se</td>
<td>Urine</td>
<td>442 ± 5 (n=4)</td>
<td>460 ± 30</td>
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</tbody>
</table>

References

FDMS and FABMS have significantly influenced the analysis of organo-metallic compounds. Usually by one or both desorption/ionization techniques molecular ions of the analytes were formed. But often mass spectra were complicated by the presence of several ions that arise by alkali metal impurities and/or by reactions with the liquid matrix. Nevertheless, the combination of the above mentioned desorption/ionization methods with tandem mass spectrometry have provided useful molecular weight and structural information (1). However, studies on fission fragment induced desorption, another "soft ionization" technique, of inorganic and organometallic compounds have been relatively scarce (2-5). Even less is known about the behaviour of metallocenes under Cf-252 fission fragment bombardment and subsequent mass spectrometric analysis (6,7). The present work focused on three objectives. The main one was to demonstrate the potential of PDMS in characterizing various ferrocenyl-phenyl-plumbanes (FPP). PD mass spectra of all FPPs showed intense M⁺ ions and only low intensity adduct ions (see Fig. 1). The second objective was to compare the molecular ion regions obtained by a linear (15 cm) and a reflecting (drift region equivalent to ca. 100 cm) TOF instrument (see Fig. 2). The resolution of the reflectron mass spectrometer turned out to be sufficient to resolve the isotopic pattern of the molecular ions (R = 1500 at FWHM). The theoretical calculated isotopic intensity distribution showed an excellent correlation with the measured distribution (correlation coefficient r = 0.9971). The last objective was to try to find significant fragment ions especially with the reflecting instrument which allow to obtain structural information. All FPPs underwent extensive fragmentation. Since these compounds contain numerous atoms with multiple isotopes, the isotopic pattern of fragment ions can be interpreted to provide compositional information. The results have given interesting new information for the synthetic chemist especially with the electrostatic mirror containing instrument as well as new insights into the desorption/ionization processes of solid thin films of metallocenes by PD.

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Figure 1. Positive ion PD mass spectrum of 1,1'-bis(triphenylylumbyl)-ferrocene recorded with a short linear TOF instrument at 17 kV.

Figure 2. Positive ion PD mass spectrum of 1,1'-bis(triphenylylumbyl)-ferrocene recorded with a reflectron TOF instrument at 15 kV.

The linear TOF instrument was made available by a grant from the Austrian "Fonds zur Förderung der wissenschaftlichen Forschung". The Science Faculty of the Odense University is acknowledged for support to the electrostatic mirror.
GAS-PHASE THERMOCHEMISTRY OF METALLOCENES AND METALLOCENIUM IONS
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The study of chemical reactions in the gas-phase leads to fundamental properties of a molecule that may otherwise be masked by solvent interactions. The investigation of gas-phase ion-molecule reactions involving organometallic compounds can be used to determine thermochemical information, including gas-phase basicities and acidities, electron affinities and ionization potentials. Direct comparison of gas-phase data to solution chemistry reveals the effect solvation has upon a reaction.

Fourier transform ion cyclotron resonance, FTICR, mass spectrometry has been used to investigate gas-phase ion-molecule reactions of a series of metallocene compounds, Cp²M, where Cp denotes η⁵-cyclopentadienyl. From the measured equilibrium constant for a charge-transfer reaction (equation 1), the free energy of the

\[ \text{Cp}_2M + R^+ = R + \text{Cp}_2M^+ \quad (1) \]

reaction can be determined. If the free energy of ionization, \( \Delta G^\circ \) of R, a reference compound is known, \( \Delta G^\circ[\text{Cp}_2M] \) can be calculated.

Using organic reference compounds previously studied by equilibrium techniques, the free energy of ionization of Cp₂V, Cp₂Mn Cp₂Fe, Cp₂Ni, Cp₂Ru, Cp₂Os, and a series of ferrocene derivatives has been determined. An equilibrium ladder (Fig. 1) shown below displays all charge-transfer reactions studied in this work. Newly determined \( \Delta G^\circ \) values for the metallocenes are shown adjacent to their molecular formula. \( \Delta G^\circ \) values for charge-transfer reactions lie adjacent to arrows with values in parenthesis indicating charge-transfer reactions involving reference compounds taken from the literature.

Temperature dependence studies have been performed in order to determine enthalpies and entropies for selected charge-transfer reaction. Because temperature dependent studies have not been investigated previously using FTICR mass spectrometry, a model reaction was studied in order to assess the reliability of measured equilibrium constants as a function of temperature. The CO/Kr reaction couple (eq 2) was chosen because the \( \Delta \Delta G^\circ \) is accessible on the FTICR-MS scale and

\[ \text{CO} + \text{Kr}^+ = \text{Kr} + \text{CO}^+ \quad (2) \]

sufficient spectroscopic data exist to facilitate statistical thermodynamic calculations of enthalpies and entropies for all neutral and ionic species involved. The experimentally derived entropy and enthalpy changes for the reaction are 3.3 ± 1.4 e.u and 0.3 ± 0.8 kcal/mol respectively as compared to the theoretical values of \( \Delta S^\circ \) = 1.42 ± 0.06 e.u and \( \Delta H^\circ \) = -0.2 ± 0.01 kcal/mol. The experimental values are consistent with statistical thermodynamic values and no anomalies were found for the equilibrium constants.

The results of selected temperature dependent charge-transfer reactions are shown in Fig. 2. The entropy of reaction for the ferrocene/N,N'-diethyltoluidine reaction couple is nearly 7 e.u. higher than previously reported by Mautner. For the Cp₂Ni/Cp₂Mn couple, the nearly 6 e.u. for \( \Delta S^\circ \) is not unexpected when the large geometric changes subsequent to ionization of Cp₂Mn are considered. From these studies, values of \( \Delta G^\circ[\text{Cp}_2M] \) were shown not to differ significantly from \( \Delta H^\circ[\text{Cp}_2M] \) values at experimental
temperatures employed. Thus the data obtained in this work can be used to obtain other useful thermochemical information.

Thermochemical cycles, (Fig. 3) were used to make estimates of average heterolytic, $\Delta H^{\circ}_{\text{het}}$, and homolytic, $\Delta H^{\circ}_{\text{hom}}$, bond dissociation enthalpies for metallocenes and metallocenium ions. Comparison of $E_{\text{a}}$ values with $\Delta G^{\circ}$ data gives differential solvation energies, $\Delta \Delta G^{\circ}$, the lower portion of the cycle (Fig. 3).

A series of ferrocene derivatives were studied in order to assess the effect substituent groups have upon $\Delta G^{\circ}$ of organometallics. The ferrocene derivatives also serve to link the upper and lower portions of the equilibrium ladder. A plot comparing $\Delta G^{\circ}$ for the ferrocene derivatives versus benzene analogues (Fig. 4) yields a straight line with slope of $\approx \frac{1}{3}$. Thus, the substituents have a greater effect on the ionization potential of benzene than for ferrocene. The proximity of the polarizing groups with respect to the reaction center generates an enhanced substituent effect for benzene than for ferrocene.

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DECOMPOSITION OF NITROSATED 4-CHLOROINDOLES.

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In Colombia, where fava beans (Vicia faba) are an important component of the diet and where nitrate intake is high, the population is at especially high risk for developing gastric cancer. Earlier reports from this laboratory detail the isolation of 4-chloro-6-methoxy-indole from fava beans (Yang et al., 1984), which upon nitrosation (under simulated gastric conditions) forms 4-chloro-6-methoxy-2-hydroxy-N-nitrosoindolin-3-one oxime, a direct acting exceedingly potent mutagen (Buchi et al., 1986). Analogous results are obtained with 4-chloroindole (I) (see Scheme 1), which upon nitrosation yields 4-chloro-2-hydroxy-N-nitrosoindolin-3-one oxime (II), which has been developed as a model for the methoxy analogue.

![Scheme 1: Decomposition Pathway for Nitrosated 4-Chloroindole](image)

At physiological (or elevated) pH, 4-chloro-2-hydroxy-N-nitrosoindolin-3-one oxime (II) was found to be unstable and gave a number of compounds (as judged by HPLC), only one of which was shown to be mutagenic to *Salmonella typhimurium* strain TM677.

$^1$H-NMR spectra of freshly purified samples of the mutagenic decomposition product (III) clearly showed the presence of 5 protons, three aromatic (two doublets and a triplet, none exchangeable) and a >CH(OH) function (two doublets, one signal lost after exchange, the other simplifying to a singlet). Notably, there was no N-H resonance (as in compound IV) indicating a N-substituted compound (with no protons on the substituent). Furthermore, in the UV spectrum there was an absorption at 386nm, consistent with an N-nitrosoamide. The NO group was thermally labile when volatised for GC-MS analysis, but the resultant mass spectrum (see spectrum A, below) was identical to that of the synthetic de-nitrosated material, 4-chlorodioxindole (IV) synthesised (with minor modifications) according to the methods of Marvel and Heirs (1941), Sadler (1956) and Marchalk (1912). Equally, compound III exhibited an identical GC-FT-IR spectrum to IV.

In samples of 4-chloro-N-nitroso-dioxindole (III) that had been allowed to stand for several hours, it was possible to see the appearance of compound V in the NMR spectrum, identified as 4-chloroisatin by comparison with NMR measurements on the synthetic material (synthesised in the preparation of IV, above). Further proof of identity was achieved by matching GC-MS and GC-FT-
IR spectra and the UV spectrum after reverse-phase HPLC purification (see spectra B and C, below). This material was also present in the crude decomposition mixture from compound II.

Nitrosation of 4-chloroindole with \(^{15}\)N-labelled nitrite with (and without) subsequent decomposition in \(^{18}\)O-labelled water clearly demonstrated that compound V retained no nitrogen from nitrite, but incorporated one oxygen from the labelled water (consistent with oxygen attack at C-3 of compound II).

It was demonstrated that compound III induces a mutant fraction of \(-1\times10^{-4}/\mu M\) in Salmonella typhimurium strain TM677 (with compound II inducing a mutant fraction of \(-1.3\times10^{-2}/\mu M\)) as determined by the method of Skopek et al. (1978). When tested, the synthetic compounds IV and V exhibited no mutagenic activity.

After human consumption of fava beans, when nitrosated indoles are absorbed into the stomach wall, it is clear that the corresponding increase in pH may cause decomposition to the nitrosated dioxindole, and ultimately to the inactive isatin. Such factors as the rates of decomposition, and the mechanisms of mutagenesis are obviously important in determining the relative carcinogenic potential of these species.

E.I. Mass Spectra of Decomposition Products.

REFERENCES
Toxicity of benzene requires the metabolism of the parent compound to one or more metabolites (Tunek et al., 1982). Benzene is mainly metabolized in the liver by the p-450 system to the reactive intermediate benzene oxide. This epoxide is very unstable and converts to several metabolites: phenol, catechol, and toxic metabolites such as hydroquinone, benzoquinone, and muconaldehyde. Bone marrow depression in humans (Aksoy, 1985) is presumably caused by the covalent binding of reactive metabolites to macromolecules in bone marrow after the benzene exposure. This study shows new ways to derivatize the urinary metabolites of mice exposed to benzene. Benzene is a petroleum by-product, and a component of unleaded gasoline. It is volatile and fat-soluble, and absorbed by inhalation, ingestion, and dermal contact. Because of its extensive use, a large number of people were exposed regularly to benzene. Benzene is mainly metabolized by the cytochrome p-450 monooxygenase system which is mostly found in the liver, and also in the bone marrow. This toxicity in humans is characterized by panleukopenia (depression of the levels of all circulating blood cell types) or one of its cytopenic variants (depression of the levels of one or more, but not all, of the circulation blood cell types). Lymphocytopenia is one of the most sensitive and easily measured indicators of benzene toxicity in the peripheral blood. Benzene metabolites, such as free phenol, hydroquinone, and catechol were detected not only in the blood but also in the bone marrow (Rickert et al., 1979).

Male B6C3F1 (n=5) were dosed a single time by gavage with 1.4 grams/Kg body weight benzene in corn oil and urine was collected at 24-hour intervals for one week. Aliquots of urine were acidified to a pH of 4.4 and hydrolyzed by &-glucuronidase/sulfatase at 37°C for 16 hours. The metabolites were extracted with 3 % MeOH/ethyl acetate using Clin Elut disposable columns. Simultaneous derivatization of hydroxyl and carboxylic acid groups were carried out by Tri-BSA agent. Mass spectra and GC retention times of TMS-derivatives were compared with purchased compounds. All spectra were obtained on a HP 5985 quadrupole mass spectrometer equipped with capillary columns (DB-1 30 meter). Helium was used as carrier gas (25 cm/sec), and both injection port and ion source were 200°C. The column temperature was programmed from an initial oven temperature of 100°C held for 1 min, with the program rate of 5°C/min, to the final temperature of 180°C held for 1 min. Concentration of each component was calculated by comparison with standards.

TMS-derivatives of phenol, catechol, hydroquinone, and muconic acid were easily prepared by one step reaction and standards were within a linear range. One of the toxic metabolites, hydroquinone was present in the highest concentration and catechol in the lowest. No metabolites were detected after 7 days. This simple method can be used to analyze the urinary metabolites of mice exposed to the benzene and its results can be correlated with the bone marrow depression.

REFERENCES

TMS - DERIVATIVES

URINE OF MICE EXPOSED TO BENZINE

HYDROQUINONE MW 254

MUCONICACID MW 286

PHENOL MW 166

CATECHOL MW 254

M/Z

966
INTRODUCTION

The structural characterization of carcinogen-modified nucleosides derived from biological (particularly human) sources has been limited because in many cases only small (subnanogram) quantities were isolated prior to mass spectral analysis. To date, metastable ion mass spectra of protonated molecule ions derived from FAB-generated, silylated amino PAH-nucleoside adducts have been acquired using as little as 4-5 ng (applied to the probe) in a targeted compound analysis mode (1). We have demonstrated here for the first time the identification of unknown PAH nucleoside adducts for subnanogram levels of sample.

EXPERIMENTAL

All FAB MS/MS spectra were acquired using a Finnigan MAT TSQ 70 triple quadrupole mass spectrometer equipped with an Ion Tech fast atom gun. Xenon was employed in the primary atom beam at energies of 8-10 KeV and Ar as the collision gas for CID. Dithioglycerol was used as a matrix. CAD spectra of the protonated molecule ions were acquired using 50 eV (constant neutral loss, CNL) and 100 eV (single and dual reaction monitoring) collision energies. For silylated adducts, derivatization was accomplished using N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) at 55° C for 1 hour.

CONSTANT NEUTRAL LOSS MS/MS

We employ CNL scans (2) to identify unknown (previously silylated) amino PAH-nucleoside adducts by scanning Q1 and Q3 simultaneously, with a mass offset corresponding to that of the neutral fragment lost in the CAD process (3). This neutral corresponds to a disilylated deoxyribose (260 amu). Since only those ions that fragment to lose 260 amu are detected, most of the chemical noise accompanying regular FAB analysis is eliminated. The CNL spectra of three model amino PAH-nucleoside adducts were obtained by adding all scans acquired (typically 25) from a single probe loading. Figure 1 shows the CNL spectrum obtained using 500 pg of N-(deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-C8-AAF). The ions at m/z 633 and 705 correspond to the protonated modified nucleoside plus 2 and 3 TMS groups, respectively. Similar results were obtained using 2 ng of N-(deoxyguanosin-8-yl)-4-aminobiphenyl (dG-C8-ABP) and 3-(deoxyguanosin-N2-yl)-2-acetylaminofluorene (dG-N2-AAF). In each case a TMS containing protonated molecule was readily observed. However, with dG-C8-ABP, ions corresponding to protonated molecules for the bis- and tris-TMS derivatives not containing a carcinogen moiety (ABP) were also observed at m/z 412 and 484.

Single reaction monitoring (SRM) was employed to determine the detection limits for the analysis of dG-C8-ABP and dG-N2-AAF using this compound-specific methodology. This was achieved by setting Q1 to the mass corresponding to the protonated molecule for the TMS-derivatized nucleoside and Q3 to the mass of the BH2* fragment resulting from loss of the bis-silylated deoxyribose moiety. In these experiments as little as 50-100 pg of modified nucleoside could be detected.

The most significant obstacle to improving the sensitivity for the analysis of all of the compounds we have studied appears to be the silylation step. The efficiency of silylation increases with reaction time, as does the extent of cleavage of the amino-PAH moiety from the nucleoside base. Further studies will be directed towards determining the best conditions for silylation without cleavage of the carcinogen from the nucleoside base.
ISOTOPE DILUTION MS/MS

Isotope dilution studies were performed to establish the linear range and limit of detection (LOD) using a model base-amino PAH adduct. The TSQ was switched between characteristic fragmentation reactions of the deuterated standard, guanine-C8-(d4-ABP) (m/z 328 to 203) and the targeted compound guanine-C8-ABP (m/z 319 to 195). These fragmentation reactions are attributed to the same cleavage in both compounds based on FAB MS and FAB MS/MS spectra from each compound. (A single H-D exchange accompanies this cleavage.) For each analysis the probe was loaded with 125 ng of guanine-C8-(d4-ABP) and variable quantities (300 pg to 6 ng) of guanine-C8-ABP. In figure 2, the fragment ion intensity ratio (expressed in percent) for m/z 195 and m/z 203 is plotted against the amount of guanine-C8-ABP. Four replicate loadings were run for each quantity of guanine-C8-ABP and 25 scans were added to obtain each point in the plot.

The least squares line in figure 2 has a slope of 9.35x10^4 and an intercept of 0.680%, suggesting the LOD is approximately 600 pg with a background of 300 pg. This background is attributed to a low level of guanine-C8-ABP in the guanine-C8-(d4-ABP) that contributes significantly when the deuterated standard is present in excess.

CONCLUSION

We have demonstrated that the analysis of carcinogen-modified nucleosides may be carried out at subnanogram levels. These data suggest that the presence of a suspected amino PAH adduct may be confirmed using SRM with as little as 50 pg of adduct. Quantification using isotope dilution has been accomplished with a LOD of about 600 pg. Lower LODs may be achieved using smaller quantities of (or more completely labeled) internal standards.

REFERENCES


ACKNOWLEDGEMENTS

We thank Dr. F.A. Beland for samples of dG-C8-AAF, and dG-N2-AAF, and Dr. F.F. Kadlubar for dG-C8-ABP. This work was supported in part by the Center for Indoor Air Research (CIAR #90-002).

FIGURE 1: FAB CNL mass spectrum from 500 pg of dG-C8-AAF.

FIGURE 2: Linear least squares fit of the relative response factors obtained using guanine-C8-ABP via isotope dilution mass spectrometry and dual reaction monitoring.
INTRODUCTION

Accumulated oxidative damage to neuronal DNA bases has been implicated in disorders of aging such as Parkinson's and Alzheimer's diseases (1). In order to test this hypothesis, we have been developing GC/MS methods for measuring thymine glycol (2). Initially, we tried to find a suitable derivative for GC/NCl of thymine glycol, but we were stymied by its base lability, thermal instability, and failure to form a compound with sensitive electron capture properties. Instead, we have focused upon its specific conversion to 2-methylglyceric methyl ester (3) following base hydrolysis, sodium borohydride (deuteride) reduction, and acidic methanolysis. This reaction affords the significant advantage that 2-methylglyceric methyl ester can be separated from intact DNA, rather than requiring the separation of thymine glycol from the other DNA bases. Sodium borodeuteride adds specificity, because monodeuteriated 2-methylglyceric methyl ester results from its use.

We had used the bis-trifluoroacetyl-2-methylglyceric methyl ester for EI-GC/MS, but we were unable to obtain sufficient sensitivity to measure basal levels of thymine glycol in native DNAs, although we could readily measure thymine glycol in intentionally damaged DNA. In order to improve detection limits, we sought a derivative which would add mass, retard volatility, direct fragmentation to structurally characteristic ions, and have stable chemical and physical properties. Several derivatization reactions were tested, and the di-t-butyldimethyl silyl derivative was found to be most satisfactory. Note that this reaction only proceeds with acid catalysis, precluding the use of commercial t-butyldimethylsilylimidazole or MTBSTFA without silylchlorides.

EXPERIMENTAL METHODS

A solution of the internal standard (d4-thymine glycol, 16 ng/10 µL) is added to each tube. Then DNA (1-2 mg in 100-200 µL TE buffer) or thymine glycol quantitative standards (0.1-to-25 ng) are added, and 100 µL of NaBD4 (10mM) in 0.2 M NaOH (prewashed with CH2Cl2) is added next, and the mixture is heated for 1 hour at 37°C. All solutions are prepared fresh daily. The reaction is quenched with 100 µL of 6M HCl and dried in a vacuum desiccator. Methanolic/HCl (200 µL, 3% by weight) is added, vortexed, and heated 30 minutes at 70°C. The samples are dried in a vacuum desiccator, and 100 µL of t-butyldimethylsilyl imidazole reagent (prepared from 150 mg t-butyldimethylsilyl chloride, 170 mg imidazole in 1 mL acetonitrile, washed five times with equal volumes of heptane) is added and heated at 70°C for 30 minutes. The products are partitioned between 100 µL heptane/decane (90/10) and 100 µL water, and the organic phase is removed after freezing the tube with dry ice. The organic extracts in autosampler vial inserts are concentrated in vacuo to approximately 20-25 µL.

Analyses were performed using a Hewlett Packard 5890 GC and 5970 Mass Selective Detector with a 30 meter, 0.25 mm i.d., 0.15 µ DB225 column programmed from 90°C (1 minute) to 180°C at 10°/minute, followed by a 40°C/minute ramp to 240°C to remove contaminants. The di-tBDMS-2-methylglyceric methyl ester elutes at ~8 minutes.
Results
The linearity of standard curves was excellent over the range 0.5-25 ng/tube, with 0.5 ng being the lower limit of detection. The chromatographic behavior of the di-tBDMS-2-methylglyceric methyl ester is excellent, but DB-225 was preferred to non-polar phases in order to provide separation from several interfering background ions. There remains a measurable interfering background ion at m/z 306 which causes a non-zero y-intercept on standard curves and limits use of this assay to lower levels. Measuring the thymine glycol in native salmon sperm or herring sperm DNA provided a measure of the applicability of these methods to known materials. The measured thymine glycol in 1 mg quantities of salmon sperm DNA (1.61 ng ± 0.41 SD) and herring sperm DNA (1.16 ng ± 0.41 SD) were consistent with and proportional to the amounts measured in 2 mg quantities (salmon sperm DNA, 4.33 ±0.76 SD; herring sperm DNA 2.35 ± 0.43 SD).

Pitfalls
In all assay development, there are unexpected results which are encountered along the way. In the DNA thymine glycol assay, we noted an apparent improvement in the yield of product from native DNAs, up to 20-40 ng/mg DNA. Because we had optimized the reaction conditions for detection of the d_{4}-thymine glycol internal standard, we were pleased. However, when we tested this observation with thymine, we determined that thymine could be oxidized to thymine glycol during the assay unless conditions were employed to prevent such an artifactual oxidation. For example, when the thymine glycol in 100 μg quantities of commercial and recrystallized thymine was quantified, if less than 1 mM NaOH base strength was used, or if the thymine was taken to dryness from a dilute base solution, then significant quantities of thymine glycol were measured. With the conditions reported above, 4.7 ng of thymine glycol were measured in 100 μg of commercial thymine, and 2.7 ng in 100 μg of the recrystallized material.

Conclusions
A significant sensitivity enhancement has been attained with respect to the methods reported previously due to several factors. First, the use of a di-tBDMS derivative has permitted sample concentration in the final step, providing a 10-15-fold improvement. Second, adoption of a DB225 chromatographic column largely eliminated non-specific background, affording lower detection limits. Thirdly, determination of optimal hydrolysis, reduction, and methylation conditions largely eliminated artifactual oxidation of thymine in native DNAs so that consistent and mass proportional yields of thymine glycol could be quantified. These levels (~1 ng/mg DNA or 1 ppm) are quite low, such that a further sensitivity enhancement of 10-to-100 fold is desirable for application of this measurement to human brain tissue.

Acknowledgments
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References
Methodologies for the Characterization of Damage to DNA Using Dynamic FAB Mass Spectrometry

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DNA adducts are formed by the covalent attachment of carcinogenic species to the base, sugar, or phosphate portion of the DNA molecule and are widely believed to play a role in the process of carcinogenesis. The techniques normally employed to characterize these species at physiologically significant levels including 32P-postlabeling, immunoassay, and fluorescence line-narrowing spectroscopy are generally not able to provide pertinent structural information for unknowns. We have explored the utility of dynamic FAB mass spectrometry in various modes for the characterization of these species with the ultimate goal of analyzing these compounds in biological matrices. Studies in high and low energy CID, negative dynamic FAB, and the utility of an interface between capillary electrophoresis and dynamic FAB are discussed below. The four adducts used for this work all involve carcinogen attachment to the base portion of deoxyguanosine and include N-(anthracen-9-ylmethyl)-deoxyguanosine, N-(deoxyguanosin-8-yl)-2-aminofluorene, N-acetyl-N-(deoxyguanosin-8-yl)-2-aminofluorene, and N-(deoxyguanosin-8-yl)-4-aminoazobiphenyl.

Instrumentation. Data were collected on two different MS systems. The first consisted of a VG70-250SE double focussing mass spectrometer employing a xenon FAB gun operated at 8 kV and 1.8 mA. This system was used for the negative ion work, the CE-CFFABMS interfacing, and the high energy collision studies. The second configuration consisted of a VG Quattro triple quadrupole system outfitted with a cesium ion FIB gun operated at 10 kV and 2.7 A. This instrument was used for acquiring the low energy CID data.

Negative Ion Spectra. Negative ion spectra were acquired using a dynamic FAB matrix solution of 5% glycerol, 5% ACN and 15% MeOH in water. Samples were dissolved in methanol and introduced via flow injection using a Rheodyne injector with a 0.5 uL loop. At a source temperature of 50 C, a flow rate of 5 uL/min through the 50 um ID fused silica capillary was employed.

Positive FAB analysis has been shown to have great utility for the characterization of PAH-DNA adducts (1). It has been found that the negative FAB spectra of these species also show provide many diagnostically useful ions and compare well with the positive ion spectra in terms of information content. In addition to giving ions which arise from the parent molecule ([M-H]-), loss of deoxyribose ([B-H]-), and cleavage through the sugar (Sj), the negative ion spectra contain at least two unique features which make this mode a useful complement to the positive mode. First, in all cases, molecular ions are enhanced relative to other ions in the spectra. This effect is most pronounced for the acetylaminofluorene adduct where the molecular ion that accounts for < 10% of the base peak in the positive ion spectrum becomes the base peak in the negative mode. A second noteworthy feature is the presence, in the C8 adduct spectra, of ions which provide useful linkage position information as they arise from cleavage through the guanine with charge retention on the PAH portion of the molecule (see Figure 1).

Negative FAB analysis was found to be somewhat less sensitive than positive for the conditions employed. It is anticipated that under more basic dynamic FAB conditions (e.g. the use of matrices such as diethanolamine or triethanolamine) greater sensitivity may be observed.

Capillary Electrophoresis Dynamic FAB MS. In an effort to develop an on-line separation/structural characterization technique for the analysis of DNA adducts, we have assembled a capillary electrophoresis-dynamic FAB MS system (Figure 2) which employs a liquid junction interface similar to those previously shown to have utility for CE-CFFABMS work (2,3). Our goal is to use this technique in conjunction with CE with UV detection to confirm the presence of target adducts and to characterize unknown adducts in reaction mixtures and biological samples. To date, the adduct N-acetyl-N-(deoxyguanosin-8-yl)-2-aminoazobiphenyl has been employed as a standard to evaluate the performance of the interface for this application. Good quality full scan spectra were acquired in the positive and negative modes for 7.5-15 ng adduct and single transition monitoring of the loss of deoxyribose from the molecular species allowed facile detection of as little as 75 pg of material (Figure 3). Capillary electrophoresis was conducted using a buffer which consisted of a 0.01M NH4OAc solution adjusted to pH 9.5 with NH4OH and a CE column that was a 50-70 cm 75 um ID fused silica capillary with polyimide coating. Injections were made by siphoning of 7.5-15 nL of the sample dissolved in methanol at the anode. CE analyses were conducted at 10-15 kV (7-11uA). The dynamic FAB matrix consisted of 1% glycerol, 5% ACN, and 19%MeOH in H2O which flowed at a vacuum induced rate of about 1 uL/min. A 65 cm 5C um ID fused silica capillary with a polyimide coating was employed as the dynamic FAB capillary.

High and Low Energy CID Spectra. High energy FAB CID spectra were taken using a VG70-250SE double focussing magnetic instrument at 8 kV using linked scanning techniques. Samples were dissolved in methanol and introduced to the source by flow injection dynamic FAB employing a matrix solution consisting of 5% glycerol, 5% ACN, and 19% MeOH in water. Collision conditions were established by the attenuation of the precursor ion to 30% with the helium collision gas.
Low energy CID spectra were acquired on a VG Quattro triple quadrupole mass spectrometer at 100 eV. Precursor ions were attenuated to 30% by the appropriate pressure of argon collision gas. The dynamic FAB matrix solution consisted of 1% glycerol, 19% MeOH, and 5% ACN in water. The precursor ions chosen for these studies represent the loss of deoxyribose from the [M + H]+ ion in full scan spectra. CID spectra of these ions have generally been found to be rich in structural information.

Since certain of the experimental parameters could not be held constant due to variations in instrument design for the low and high energy systems (different dynamic FAB probes, different FA(I)B guns, etc.), a comparison of high and low energy CID sensitivity is not straightforward. However, it was found that the triple quadrupole spectra were (as expected) cleaner and easier to interpret than the linked scan data. This is due both to the faster scan times (an advantage when spectra are being taken across an eluting peak) and the resolution of precursor selection in the low energy system. Conversely, while the low energy spectrum of the methylanthracene adduct is relatively featureless, the high energy spectrum shows an increase in the number of structurally significant peaks (Figure 4).

This work was supported by grants from HEI and the EPA.

IDENTIFICATION OF POLYCYCLIC HYDROCARBON DIOL EPOXIDE-HEMOGLOBIN ADDUCTS FROM HUMAN SUBJECTS BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

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This research seeks to establish a method for identification and quantitation of polycyclic tetrols which are released upon hydrolysis from polycyclic diol epoxide-hemoglobin and/or DNA adducts. Since adduct levels in hemoglobin are very low (femole to pmole per gram of globin) a very sensitive method of detection is required. At present the best approach is derivatization and use of GC-MS.

Chess et al. [1] have reported the mass spectra of permethyl, peracetyl and per(trifluoroacetyl) derivatives of benzo[a]pyrene tetrol under three different ionization modes (EI, PCI and NICI). A substantial number of fragment ions are produced under the three ionization modes, which makes these derivatives unsuitable for quantitation. We have studied the mass spectra of TMS derivatives of several different polycyclic tetrols. TMS ethers have been the best derivatives for quantitation of polycyclic tetrols under NICI (methane) conditions.

As shown in Figure 1, TMS derivatives of tetrols (e.g. 6-methylchrysene tetrol) tend to fragment extensively under EI and PCI conditions, whereas under conditions for NICI (e.g. chrysene tetrol and 6-methylchrylene tetrol), they give a single dissociative electron capture fragment, (Figure 2) which corresponds to M-(TMS-O-TMS)-. One major drawback to the TMS derivatives of tetrols is that they are unstable. As shown in Figure 3, for 1,2,3,4-tetrahydroxy-1,2,3,4-tetrahydrochrysene, the signal is reduced seven-fold when the derivatized sample is stored overnight at -20 °C; no signal can be detected from an identical sample after storage for 4 hours at room temperature. This behavior is even more pronounced in biological samples (detectable lifetime of less than 20 minutes), which makes it virtually impossible to obtain duplicate chromatograms of a biological-derived sample.

We have begun using a new method in which derivatization takes place in the injection port of the gas chromatograph. Reproducibility using this derivatization method is excellent as shown in Figure 4. As long as the underivatized sample is stable in a solvent compatible with the derivatization reagent, such as acetonitrile, this method is satisfactory. Figure 5 shows two gas chromatograms where the two different derivatization methods discussed above were used on the reaction products of (±)-trans-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene and ethanol. The two methods of derivatization are comparable with respect to relative peak intensities. Total signal is much higher using the new method (the sample mixed and derivatized with N-methylsilylimidazole/pyridine in the GC injection port) than that seen after using the old method (sample mixed with N-methylsilylimidazole/pyridine in vial).

All of the analysis were done with a HP 5989 MS and a 5890 GC inlet system. Good separation was obtained for the TMS derivatives of polycyclic tetrols (benzo[c]phenanthrene tetrol, benz[a]anthracene II tetrol chrysene-tetrol, 5-methyl- and 6-methylchrysene tetrols) using a 30 m DB-17 fused-silica (J&W Scientific GC column).

In conclusion, the new method of derivatization is the best solution to the quantitation for selected ion monitoring of the dissociative electron capture fragments from perTMS derivatives of benzo[a]pyrene tetrahydrotetrols under the negative ion chemical ionization mode, as well as the identification of the other tetrols which are released upon hydrolysis of hemoglobin and DNA adducts.

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SELECTED ION MONITORING SHOWS THE SIGNAL REDUCTION FROM STORAGE OVERNIGHT AT -30°C AND COMPLETE LOSS OF SIGNAL IN THE PRESENCE OF THE AG 3 AFTER FOUR HOURS AT ROOM TEMPERATURE.

SELECTED ION MONITORING SHOWS REPRODUCIBILITY OF THE NEW METHOD AFTER THE SAMPLE WAS STORED AT -30°C OVERNIGHT AND 4 HOURS AT ROOM TEMPERATURE.

COMPARISON OF THE TWO METHODS ON THE REACTION PRODUCTS OF 6-MTH DII. SODIUM OF RENIFOLAPHINE AND ETHANOL.
INTRODUCTION: Over eighty different natural nucleosides are presently known in nucleic acids, the greatest number being in tRNA. Because new nucleosides from these sources are usually isolated in small amounts, MS/MS plays a potentially important role in the identification of submicrogram amounts without isolation. Hence, experiments have been undertaken to assess the utility of trimethylsilylation coupled with tandem mass spectrometry for the structure analysis of nucleosides in mixtures. The extra step of derivatization adds increased effort and the uncertainty of uniform reaction yield; however, in many situations, the advantages outweigh the disadvantages, as follows: (1) CID generally produces more extensive and informative fragmentation of the silylated nucleoside compared with the underivatized compound, and a wealth of structure assignment data from EI-MS studies can be readily used.

(2) Silylation results in greater dispersion of molecular weight values for most nucleosides, and shifts them to a higher and more interference-free region of the spectrum for MS-1 mass selection. (3) In the case of the FAB-TMS approach, a significant increase in sensitivity is obtained due to surface activity conferred by the silyl group in the FAB ionization step.

EXPERIMENTAL: 20 μg of dried sample was added to a 20 ul mixture of N,N-bis(trimethylsilyl)acetamide (BSA), trimethylsilylchlorosilane (TMCS) and pyridine (100:1:10) and heated for one hour at 105° C. Transfer RNA* (25 μg) was hydrolyzed to nucleosides by a standard procedure using nuclease P, and bacterial alkaline phosphatase. The dried digest was silylated as described above. All mass spectra were acquired on a VG 70SEQ Instrument. FAB spectra were derived from 1 μg sample added to 1 ul of 3-NBA.

RESULTS AND DISCUSSION: Fig. 1 illustrates the CID spectrum of guanosine-TMS5 M+*. In general, the EI-MS of trimethylsilyl nucleoside derivatives exhibit three series of ions:

(1) molecular ion series: M-15, M-90, M-105, M-180; (2) base series ions: B+1, B+2, B+30, B+58, B+74, B+116, B+132, B+204, B-14; and (3) sugar series ions: S-H, S-90, S-105, S-116, S-180, m/z 217, 169, 103. The same pattern is exhibited by the CID of M+* but with a considerable difference in the abundances of ions. The silyl ions m/z 73 (base peak), 147, and 217, which are structurally uninformative, are usually more abundant in the primary spectrum and are reduced in intensity manifold in the CID spectrum. The structurally more diagnostic base series ions are significantly increased in abundance. The CID spectrum of even electron ion (M-CH3)+ produced less fragmentation when compared to M+* with the predominant ions being those missing a silyl methyl e.g., m/z 352, 260. MS/MS studies with 15CD3 in the base and 2H3 in the sugar of uridine-TMS4 shows that B+58 is predominantly formed from (M-CH3)+ in the unlabeled derivative instead of the alternative precursor B+74.

Fig. 2 shows the CID spectrum of guanosine-TMS5 MH+ ion. This spectrum exhibited two intense ions: BH+ and m/z 259; BH+ is formed by base protonation followed by glycosidic bond cleavage. The m/z 259 ion is also present in all U, C and A TMS derivatives and is therefore a sugar fragment ion (S+TMSOH). Its intensity is reduced significantly in 2'-O-methyluridine-TMS3 and an abundant new peak appears at m/z 201 (S*-CH2OH). However, this effect is reversed in 3'-O-methyl nucleosides, suggesting that the 2'-O substituent is lost preferentially in the formation of m/z 259, providing a means to distinguish 2'- and 3'-substitution in nucleosides. A simple mechanism can be envisaged for its formation by the loss of BH and TMSOH from MH+ (Scheme 1).

IDENTIFICATION OF MODIFIED NUCLEOSIDES IN tRNA: An application of FAB-MS/MS, with silylation as described above, applied to one μg of crude enzymatic digest of E. coli tRNA, is shown in Fig. 3. The assignments shown indicate side chain-related ions which are highly improved over those produced for underivatized nucleosides by EI, FAB or thermospray ionization. The quantity of nucleoside Q detected in this experiment is estimated as 10 ng, an amount 5 to 10 times lower than without silylation. Further studies of applications involving RNA hydrolysates are in progress.
REFERENCES

FIG. 3: CID spectrum of Queuosine-TMSS MH⁺ ion from t-RNA*!” digest

FIG. 2. CID spectrum of guanosine-TMS5 MH⁺ ion from t-RNA*!” digest

FIG. 1. CID spectrum of guanosine-TMS5 MH⁺

Scheme 1

R = TMS, Me (or isomer)
Determination of Unique Oligonucleotide Composition from Molecular Mass.

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Recent advances in ionization techniques and technologies available to the biomedical mass spectrometrist have brightened the prospect for mass measurement of structurally significant nucleic acid oligomers. Electrospray and matrix-assisted laser desorption methods have successfully demonstrated the production of large gas-phase ions for mass analysis. Several researchers have recently reported [1,2] the mass measurement of intact tRNA molecules (Mr ~25,000). In contrast to peptides and proteins, the preponderance of nucleic acids are composed of only four structural elements. The current work focusses on the possibility of treating a mass measurement of a nucleic acid oligomer for determination of base composition in a manner analogous to the elemental composition determination of small organic molecules by measurement of exact mass. Unforseen results were obtained in which it was found that ±1 Da accuracy in molecular mass measurement allows substantial reduction in compositional (A, U(T), C, G) possibilities, often to a unique value.

The magnitude of the problem was investigated by formulating software to exhaustively calculate the molecular weight and composition of all possible DNA and RNA oligomers up to 100 bases long. It is readily apparent from Figure 1 that a mass measurement accurate to only 1 Da is insufficient to uniquely define the composition of an oligonucleotide with an Mr greater than ~2500, when there are no constraints on the composition (DNA curve). This limit arises from mass redundancies resulting from specific linear combinations of certain nucleotides. For example, the oligomers C2G3, Mr = 1584.031, and A5, Mr = 1584.083, Δm = 0.052 u, are the first such redundancies. The ability to distinguish these two compositions based on mass measurement is just barely within the current state of the art, using magnetic sector instruments [3]. Typical resolution requirements for the unambiguous base composition determination are 10 ppm at mass 5,000, and 0.4 ppm at mass 10,000. These requirements are far in excess of the capabilities demonstrated to date for quadrupole mass analyzers with electrospray ion sources.

When additional information about the oligomer is available (e.g. the number of residues), then the uniqueness of a mass measurement may be extended to larger molecules. This is due to the elimination of certain mass redundancies from the overlap of pyrimidine rich oligomers of length n+1 with purine rich oligomers of length n. Note that although the number of potential candidate compositions is reduced by the structural constraint (number of residues), the mass accuracy required to differentiate them remains the same. However, at lower masses, more significant reductions are possible where length overlap occurs, often reducing the candidate compositions to one.

When the composition of an oligonucleotide is constrained in any dimension, then the measured mass uniquely defines the composition in increasingly larger oligomers. For example, enzymatic digestion of RNA with RNase T1 yields ribonucleotides with a single G, at the 3' terminus. This compositional constraint of one and only one G eliminates any mass redundancy up to 4640 u, and succeeds in reducing the number of
possible compositions by a factor of 10 (Figure 1, RNA curve). This principle is presently being used as part of a strategy to identify and locate modified nucleotides in large ribosomal RNAs [4].

![Diagram](image)

Figure 1  The average number of compositions per 1.0 u mass interval. The DNA curve is unconstrained data, while the RNA curve is compositionally limited such that each oligomer has only one G residue. Data points were computed by counting the number of possible oligomers within a 100 u interval, centered about selected masses.

References


Acknowledgement

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The 39th ASMS Conference on Mass Spectrometry and Allied Topics

FAB MS and MS/MS of TMS Nucleoside Derivatives; Comparison of Low vs. High Energy CID Spectra for Positive / Negative Ions.

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Introduction

The EI mass spectra of adenosine-TMS₄ (Ado-TMS₄) and uridine-TMS₄ (Urd-TMS₄) have been studied¹,² and showed a large number of structurally significant fragment ions, including a weak [M]+ ion. The FAB mass spectrum of Ado-TMS₄ contained³ numerous structurally related fragment ions and a strong [MH]+ ion. The FAB spectrum of free adenosine (Ado), on the other hand, contained few fragment ions but a strong [MH]+ ion. Consequently, formation of TMS derivatives of nucleosides increases the amount of structural information from the FAB mass spectrum. Difficulty in identifying structurally important ions occurs, however, because of interference from matrix ions and the presence of ions from both the TMS₄ and TMS₃ (hydrolysis product) forms of the nucleoside.

Mass spectrometry-mass spectrometry (MS/MS) is not subject to the above mentioned problems of interference or ambiguous ion assignment, and has been used to generate daughter ion spectra of Ado-TMS₃ (negative ion) and Urd-TMS₃ (positive ion). A comparison is made between the low-energy CID mass spectra obtained using an EBqQ hybrid instrument versus high-energy CID spectra from a BEBE four-sector mass spectrometer.

![Ado-TMS₃ and Urd-TMS₃](image)

Experimental

The low-energy CID studies were performed on a VG 7070 EBqQ mass spectrometer with FAB ionization. m-Nitrobenzyl alcohol (NBA) was chosen as the matrix for both Ado-TMS and Urd-TMS. Unit resolution was selected for both parent and daughter ions. Daughter ions were formed in the first quadrupole at a collision energy of 70 volts with argon as the collision gas. Daughter ions were detected after the second quadrupole.

The high-energy CID studies used an AMD custom built four-sector instrument with LSIMS ionization. The matrices selected were NBA for Urd-TMS and tetraethylene glycol for Ado-TMS. Resolution of parent ions was 1000 (10% valley definition) and 200-300 for daughter ions. Production of daughter ions between MS-1 and MS-2 was enhanced by collision with Ar at 5925 volts in a collision cell. The high energy collision process required that MS-2 be scanned in a B/E=constant linked scan mode.

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Results

A. CID Daughter ions of Urd-TMS3 [MH]+ observed:
[C3H3O2TMS]+, [S-2TMSOH]+, [CH2OTMS]+
[S-2TMSOH]+

B. CID Daughter ions of Ado-TMS3 [M-H]- observed:

Discussion

Comparison of low- and high-energy CID results are limited to the total numbers of ions observed in each mode of analysis and the types of ions present in the mass spectra, i.e. ions belonging the molecular ion related (M) base related (B) and sugar related (S) series. Comparison of relative intensities of the ions is not meaningful because of software differences between the EBqQ and BEBE instruments.

The low-energy CID positive daughter ion spectrum of Urd-TMS3 contains ions derived from the M-series, B-series and the S-series. The high-energy CID daughter spectrum contains essentially the same number and types of ions observed in the low-energy spectrum, with both mass spectra being dominated by S-series ions—as reported for the EI mass spectra of pyrimidine-TMS nucleosides.

The low- and high-energy CID negative daughter ion spectra of Ado-TMS3 show the same degree of fragmentation reported for the negative EI mass spectrum of Ado-TMS3. In addition, S-series ions are not observed in either low- or high-energy daughter ion spectra, consistent with the negative EI normal spectra.

Conclusions

The positive daughter ion spectrum of Urd-TMS3 contains numerous structurally significant fragment ions similar to the normal EI and FAB spectra of Urd-TMS3, but without the ambiguity and interferences observed in FAB.

The low- and high-energy CID negative daughter ion spectra of Ado-TMS3 contain the same fragment ions. Consistent with conventional spectra, the negative ion CID results reveal much less fragmentation than the corresponding positive ion studies.

The low- and high-energy CID positive daughter ion spectra show no significant difference in the fragmentation of Urd-TMS3.

References


Support for this work by the National Institutes of Health grant CA43608 is gratefully acknowledged.
As a compound class, oligonucleotides have proved to be particularly difficult to ionize intact. For this reason, the analysis of this important compound class by mass spectrometry has not kept pace with that of other important biopolymers, such as peptides and proteins. However, oligonucleotides have recently been shown to be amenable to ionization by both matrix assisted laser desorption (1) and by electrospray (2). These developments provide new opportunities for the analysis of oligonucleotides by the host of mass spectrometric techniques developed in recent years for large polyatomic ions. In particular, we have been applying the quadrupole ion trap with its many capabilities for ion analysis, to large multiply charged ions formed by electrospray.

In this paper we describe the results of a study of multiply charged anions formed from several small (n=4-8) oligonucleotides carried out using a quadrupole ion trap. The focus of this work was the behavior of the anions under ion trap collisional activation conditions to explore the potential applicability of MS/MS and MS^n to multiply charged oligonucleotide anions.

All experiments were performed with a Finnigan-MAT ion trap mass spectrometer modified to allow ions formed external to the ion trap to be injected. Details of the instrument as it is used to perform experiments with electrospray have been reported (3-5). Analyte solution concentrations in 9:1 HPLC methanol/water fell within the range of 40-100 fmol/µL except for that of the hexamer 5'-d(ATGUAT)-3' which was 2 pmol/µL. All solutions were admitted by direct infusion at a flow rate of 1 µL/min with the syringe needle held at a potential of 3000--5000 V.

Anions derived from 5'-d(AAAA)-3', 5'-d(ATGUAT)-3', 5'-d(CGGATCCG)-3', 5'-d(GTGGCCAC)-3', and 5'-d(GGACGTCC)-3' were subjected to collisional activation under various conditions. Figure 1 provides illustrative data showing the MS/MS spectrum and MS^3 spectrum of the (M-5Na^+) ion from 5'-d(ATGUAT)-3'. The nomenclature used to label the ions in the spectra is illustrated in Scheme 1. The results from this study can be summarized as follows:

- Multiply charged parent anions give high MS/MS efficiencies (>50%).
- Both complementary ions from a cleavage involving charge separation are observed under gentle collisional activation conditions.
- Under relatively violent collisional activation conditions, sequential fragmentation occurs which complicates product ion charge state determination.
- There is a very strong tendency to form the adenine anion and its complementary ion as the lowest energy dissociation channel in oligonucleotides containing adenosine. There is a weaker tendency to form the guanine anion. No anions of the pyrimidines were observed.
- After loss of the adenine anion, there is a very strong tendency for cleavage of the 3' C-O bond of the sugar from which the adenine was lost.

These observations lead to the conclusion that the application of MS/MS techniques to multiply charged anions derived from small oligonucleotides should be particularly useful. The high MS/MS efficiencies observed and the tendency to yield complementary ion pairs is particularly conducive for MS^n experiments and the interesting fragmentation behavior suggests that much useful structural information can be obtained.

REFERENCES

Figure 1

Scheme 1

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The ANALYSIS OF OLIGONUCLEOTIDES AND THEIR PHOSPHORAMIDATE ANALOGUES
BY LSIMS MASS SPECTROMETRY.
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Oligonucleotides, and chemically modified oligonucleotides are of therapeutic interest. As early as 1978 the concept of "Anti-Sense" oligonucleotides was put forward to allow intervention in cell processes in order to inhibit gene expression in cells carrying abnormal genetic material. Typically synthetic oligonucleotides and their modified analogues are characterised by polyacrylamide gel electrophoresis, ion exchange HPLC, reversed phase HPLC, CZE and base composition analysis. The first gives an idea of molecular weight and to some extent purity, the following three indicate purity, and the latter is an averaging technique.

It was proposed that mass spectrometry could provide backup to statements on purity and molecular weight, moreover it could uniquely define sequence.

Oligonucleotides by their very nature have not readily lent themselves to mass spectrometric analyses. They are very polar, thermally labile materials, exhibiting poor surface activities (and therefore poor FAB qualities).

We have drawn from spectra of the following materials:
1) 5'TTT-TTT-TTT-TTT-TTT 3'
   where * denotes a modified phosphoramidate linker lie.

2) 5' TTT - TTT - TTT - TTT - TTT 3'

3) Synthetic 15-mer Oligonucleotide containing the bases C, G and T.

The -ve LSIMS spectrum of the modified oligonucleotide (1 above) is shown in Fig. 1. It shows good molecular weight information and provides bidirectional sequence along the whole molecule. The 3' phosphate sequence ions are more intense than those of the 5'. The unmodified oligonucleotide (2) gave a very poor LSIMS spectrum exhibiting a molecular ion in +ve mode only, and no sequence ions though this may well be due to an unfavourable (NH$_4^+$) counter ion. These observations accord with the findings of Grotjahn et al. The 15-mer material (3) was analysed as an Et$_3$NH$^+$ salt, and this material gave molecular weight and bidirectional sequence data (Fig. 2).

Figure 1; -VE LSIMS Spectrum.
PMY Beam: Cs$^+$ @ 30keV.
Solution In MeOH/H$_2$O
Thioglycerol Matrix.
Recorded on Finnigan MAT 900.

FULL SCAN SPECTRUM 5'TTT-TTT-TTT-TTT-TTT 3' (LSIMS, -ve)

Inherent in producing antisense oligonucleotides, are chemical modifications aimed at modifying the phosphodiester bridges to confer stability to extra and intracellular enzymes. Unfortunately modification of each internucleotide phosphate can introduce a new chiral centre, and the diastereomer mix produced can give rise to a complex chromatographic pattern (Fig. 3).
Information can be gleaned from the mass spectrum as to the presence of truncated species, this data may not be readily obtained from the chromatogram if diastereomeric components overlay truncated impurities. Generally molecular ions of truncated oligonucleotides fall at different masses from the phosphate sequence ions.

Conclusions.
As an ionisation mode LSIMS provides sufficient sensitivity to provide good molecular weight data on modified and unmodified synthetic oligonucleotides. LSIMS however has an important advantage over ionisation techniques such as Electrospray Ionisation in that it also produces sequence information, though is probably less sensitive for unmodified oligonucleotides. Judgements can be made from the spectrum regarding the presence of truncated oligonucleotides. In the case of unmodified oligonucleotides the quality of the spectrum seems heavily dependant on the nature of the counter cation. Modified oligonucleotides carry an overall reduced net charge, and are readily desalted - as such they are less prone to these effects.

Instrumentation and Methods.
All materials were dissolved in MeOH/H$_2$O and inoculated into a Thioglycerol matrix. Secondary ion spectra were derived from bombarding this matrix/analyte mix with 30 KeV Cs$^+$ primary ions. The spectra were recorded on a Finnigan MAT 900 forward geometry double focussing mass spectrometer fitted with a multi channel array detector.

References.

Acknowledgements.
The authors would like to thank A.J. Pipe and S.A. Noble of the Medicinal Chemistry Department, Glaxo Group Research, for their collaboration in providing samples and synthesising analogues used in this poster.
CHARACTERIZATION OF MANDUCA SEXTA JUVENILE HORMONE ESTERASE PEPTIDES USING CONTINUOUS-FLOW FAST ATOM BOMBARDMENT MASS SPECTROMETRY.

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Juvenile hormone (JH) esterase, an enzyme important in insect development and a potential target for selective chemical control of insect pests, was purified to homogeneity from hemolymph of fifth larval stage day three Manduca sexta using a trifluoromethylketone affinity column (1). Purified JH esterase was shown to be a glycoprotein based on its reaction with the lectins concanavalin A and peanut agglutinin, but not wheat germ agglutinin (2). Derivatization of the enzyme with digoxigenin, immobilization on nitrocellulose by dot blot transfer, and then visualization by reaction with an alkaline phosphatase-linked antibody to digoxigenin produced a positive response indicative of the presence of vicinal diols found in glycoproteins (3). Attempts to release the carbohydrate moiety by either chemical or enzymatic methods failed. Trypsinization was possible only in the presence of 6 M urea for extended periods of time up to 72 hours using immobilized trypsin (4). A peptide map was prepared using LC-MS which will be used in future studies to sequence the active site and further characterize the carbohydrate moiety.

Purified JH esterase was electroblotted from a SDS-polyacrylamide gel onto nitrocellulose for the purpose of lectin binding studies. Circular dichroism was carried out on a JASCO J-600 spectropolarimeter at room temperature and the resultant spectrum was adjusted for background. Reduced and carboxymethylated JH esterase was dissolved in 50mM Tris-HCl, pH 8.0, 1 mM EDTA, 6 M urea, and incubated for 48-72 hours at 37 °C with immobilized trypsin.

A 10 min. 100% aqueous wash on a Vydac C18 analytical column removed the urea present in the tryptic digest and was followed by a 30 min. linear gradient from 100% water to 100% acetonitrile with 0.1% trifluoroacetic acid in both solvents. The flow rate was 1mL/min. and peptides were detected at A214nm. All tryptic peptides were collected in one fraction, concentrated to dryness and dissolved in 90% H2O, 10% acetonitrile, 0.1% trifluoroacetic acid, and 0.5 % glycerol (v/v/v/w). LC-MS separations were carried out using an Applied Biosystems Model 140A dual syringe pump and Vydac C18 HPLC column (2.1mm x 15cm). The solvent system consisted of a gradient from 90:10:0.1:0.5 to 10:90:0.1:0.5 (v/v/v/w) of water/acetonitrile/trifluoroacetic acid/glycerol over 30 minutes at a flow rate of 70 µL/min. The flow was split so that approximately 7 µL/min flowed to the mass spectrometer. Mass spectra were obtained using a JEOL HX110HF double focusing mass spectrometer equipped with a DA5000 data system and trit-FAB version of a continuous-flow FAB (CF-FAB) LC-MS interface.
The lectins concanavalin A and peanut agglutinin, but not wheat germ agglutinin reacted with JH esterase indicating the presence of D-glucose, D-mannose, and galactose (1-3) N-acetyl-D-galactosamine and the absence of tri-N-acetyl-D-glucosamine. The circular dichroism spectrum of JH esterase showed the following secondary structural features: 46.0% α-helix, 6.5% anti-parallel β-sheet, 3.0% parallel β-sheet, 19.5% β-turn, and 25.0% unidentifiable. Tryptic digestion of JH esterase required 6.0 M urea and 48-72 hours at 37 °C. Reversed phase HPLC analysis with uv absorption detection showed a series of poorly separated hydrophobic peptides. Positive ion CF-FAB LC-MS analysis of the tryptic digest of JH esterase revealed the presence of more peptides than were expected based on uv absorption detection alone. Figure 1 shows examples of the protonated peptide molecules detected in the tryptic digest of JH esterase during CF-FAB LC-MS analysis. Figure 2 shows an example of the positive ion CF-FAB mass spectrum of the ion at m/z 705 in the LC-MS analysis shown in Figure 1.

COMPARISON OF B/E LINKED SCANS, MIKES, AND Q-DAUGHTER SCANS FOR OBTAINING STRUCTURAL INFORMATION FROM COMPLEX OLIGOSACCHARIDES.

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Complex oligosaccharides obtained from glycoproteins can be examined by mass spectrometry using ionization methods such as FAB, LSIMS and FD. However, not all of these techniques produce sufficient fragmentation for structural elucidation and, furthermore, problems in interpretation can be encountered when mixtures are present. This paper describes the use of linked-scan and MS/MS techniques on a hybrid instrument for obtaining the required structural information.

The compounds examined were commercial bi-, tri- and tetraantennary N-linked oligosaccharides containing 9, 11, and 13 monosaccharide residues respectively. They were permethylated (1) and LSIMS (Cs) spectra were obtained from 0.1 μg (about 50 pmole) samples dissolved in a 1:1 glycerol:thioglycerol matrix using both VG ZAB-VSEQ and AutoSpec-EQ instruments. B/E linked-scan data and MIKES spectra were obtained under normal conditions; Q-daughter spectra were obtained using xenon as the collision gas.

All three oligosaccharides (MW = 2047.05, 2496.2, and 2945.5, 12C-isotope) gave fragment ions by direct LSIMS and by the three CID techniques. Fig. 1 shows the LSIMS spectrum of the biantennary oligosaccharide and illustrates the production of major fragment ions produced by cleavages adjacent to most of the monosaccharide units with the major ion representing loss of GlcNAc from the reducing terminus as shown in Fig. 2 for the same biantennary oligosaccharide as that shown in Fig. 1. The spectrum is cleaner than the LSIMS spectrum as the ions due to the by-products of methylation are missing. Less fragmentation was present in the spectra of the larger sugars but major ions were still the result of cleavages adjacent to the GlcNAc units.

Fig. 1. LSIMS spectrum of the permethyl derivative of a biantennary oligosaccharide.
Fig. 2. B/E Linked-scan spectrum of the molecular ion of the biantennary oligosaccharide from Fig. 1.

Q-Daughter spectra were very similar to the linked-scan spectra although the ion abundance was slightly lower. Even so, spectra were obtained from all three sugars; that from the tetra-antennary sugar is shown in Fig. 3.

Fig. 3 Q-Daughter spectrum of the molecular ion of a tetraantennary oligosaccharide (per-Me derivative).

The MIKES spectra tended to be less informative, although fragmentation was, again, similar. B/E linked-scan data were also obtained from several daughter ions and yielded sequence information.

STRUCTURAL CHARACTERIZATION OF THE HOST SPECIFIC SIGNAL OLIGOSACCHARIDES OF \textit{R. leguminosarum} BY SFC AND FAB-MS

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INTRODUCTION

Higher Organisms are unable to convert atmospheric $N_2$ into biochemically useful organic nitrogen. For this, we depend on certain species of algae and bacteria. Bacteria of the genus \textit{Rhizobium} are able to infect the root hairs of specific leguminous plants, leading to the formation of nodules in which nitrogen fixation occurs.

Selected genera are able to infect only a small number of plant species, and this host specificity appears to be dictated by a set of bacterial genes, called nodulation (\textit{nod}) genes, because their influence on this step in the infection process.

These genes are activated by a stimulatory signal from plants. The \textit{nod} genes code for a series of proteins that give rise to an oligosaccharide structure which is secreted by the bacteria, causing the process of infection to begin in specific host plants. It is this specific bacteria-to-plant signal which ultimately determines host specificity.

Differences in the occurrence and expression of the \textit{nod} genes regulate the structure of the host specific signaling molecule. The host-specificity signal of \textit{R. leguminosarum}, as well as several mutant strains containing altered \textit{nod} genes, has been isolated and it is the goal of this study to examine the function of certain \textit{nod} proteins and the structure of the oligosaccharides produced by this system.

RESULTS

The oligosaccharide products of wild type and mutant \textit{R. leguminosarum} strains have been isolated and characterized by mass spectrometry, in order to determine their structures and the role of the \textit{nod} genes in the signal's biosynthesis. In order to approach this problem, glycans isolated by HPLC were analysed by FAB-MS to determine molecular weight and sequence, SFC and SFC-MS were utilized to profile the oligosaccharides, and Glycosidic linkage was determined by methylation analysis.

The fully active molecule isolated from wild type \textit{R. leguminosarum} (Figure 1) consists of five (1-4)-linked glucosamines, all N-acetylated except the terminal residue. This residue is substituted with an N-linked fatty acid, and an O-linked acetyl group. The linkage position of the acetate has not been conclusively determined. FAB-MS was used to determine to which residue of the oligosaccharide chain these substituents are bound. Figure 2 is the positive ion FAB spectrum of a precursor molecule containing one less GlcNAc than the fully active material. Analogous spectra are observed for the entire series of compounds.

The \textit{nod} genes present in \textit{R. leguminosarum} include \textit{NodABC}, \textit{FE}, and \textit{L}. By determination of molecular weights for oligosaccharide signals from mutants in which the \textit{nodL} and/or \textit{nodFE} genes are deleted, some insight into the function of these genes can be obtained. It is evident that the \textit{nodL} gene product is responsible for O-acetylation of the molecule, because deletion of this gene results in a decrease in molecular weight of 42 amu. Similarly, deletion of the \textit{nodFE} sequence results in a change of 64 amu in molecular weight. This difference can be traced to the fatty acyl residue on the terminal glucosamine.

Figure 3 is the SFC-FID profile of a mixture of glycans from the wild type \textit{R. leguminosarum} after permethylation. The profile showing 4 major peaks is consistent with results from HPLC, and SFC-CIMS provides abundant molecular ions of the expected products. Because of the difficulties inherent in purifying individual components from these systems, as well as the variety of subtle structural motifs, this is a potentially valuable tool for the rapid characterization of this class of glycans.
+FAB-MS of S3a

SFC of Permethylated Oligosaccharides from Wild Type *R. leguminosarum*
OXYGENATION OF POLYUNSATURATES IN RAT BRAIN

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Hydroxylation of polyunsaturates has been reported in various regions of rat brain and many of these hydroxy products are known to possess important biological activities. Lipoxygenation, cyclooxygenation, cytochrome P-450 dependent epoxidation or peroxidation could possibly be responsible for their formation. However, brain hydroxylation seems to occur mainly by peroxidative processes, as evidenced by the racemic stereochemical purity of the products as well as the inhibitor profile. This finding contradicts previous reports concerning leukotriene production, which suggested that 5-lipoxygenase activity exists in the rat brain. In order to determine whether leukotrienes could instead be formed by peroxidation, leukotriene B_{4} production was examined by thermospray LC/MS after pentafluorobenzyl derivatization. We also examined production of hydroxy compounds in various brain regions including cerebrum, cerebellum, hypothalamus, thalamus, pituitary and pineal body.

Male Sprague-Dawley rats were anesthetized with chloral hydrate (300 mg/kg, i.p.) and the brains were perfused with oxygen saturated saline containing heparin. After decapitation, the pituitary, pineal body and brain were quickly removed and the forebrain was further dissected into various regions. Each part or region of the brain was either homogenized or sliced and incubated with 35 μM radiolabeled or non-labeled arachidonic acid (20:4w6) or docosahexaenoic acid (22:6w3) in the presence or absence of 2 mM CaCl_{2} and 2 μM of the calcium ionophore A23187. In order to prepare reference hepoxilin-like compounds, porcine leukocyte 12-lipoxygenase was incubated with 20:4w6 or 22:6w3. Reaction was terminated by acidification to pH 3 and metabolites were extracted with dichloromethane, dried and redissolved in methanol in order to inject into HPLC/UV/radioactivity detector or HPLC/UV/thermospray MS.

For leukotriene measurement, metabolites were extracted with ethyl acetate or by solid phase extraction using a C-18 cartridge according to Powell (1) in the presence of 4 ng of 13C_{2}-LTB_{4}, then derivatized to pentafluorobenzyl esters. However, when M-PFB ions were measured by Nl/thermospray MS, no LTB_{4} production was observed above the 500 pg level, while non-perfused pituitary produced ng levels of LTB_{4}. This indicated that the observed 5-lipoxygenase activity was a result of blood cell contamination.

Among the regions examined, only the pineal body produced significant levels of lipoxygenase products. Both arachidonic acid (20:4w6) and docosahexaenoic acid (22:6w3) were metabolized to the corresponding 12- and 15-lipoxygenase products when incubated with pineal body homogenate. As shown in Figure 1, both 17- and 14-hydroxy 22:6w3 (HDHE) were produced by the pineal body. Their thermospray spectra contained M-NH\textsubscript{4}+, M+NH\textsubscript{4}+-H\textsubscript{2}O and MH+-H\textsubscript{2}O at 362, 344 and 327, indicating they were monohydroxy compounds. Their retention times coincided with those of the previously characterized 17- and 14-HDHE which were prepared by the reaction of 22:6w3 with soybean and platelet lipoxygenases, respectively. In addition to these monohydroxy products, variable amounts of a 22:6w3 metabolite was observed at a retention time between 26 and 27 min. This compound eluted at the same retention time as the epoxy,hydroxy-22:6w3 (hepoxilin-like compound) produced by the porcine leukocyte 12-lipoxygenase (Figure 2). Thermospray LC/MS spectra indicated that this compound was 13,14-epoxy,hydroxy-22:5w3, as the cleavage of the epoxidated C-C bond, which is typical for epoxidated compounds, produced fragment ions at m/z 214 and 197 as shown in Figure 3. Biological effects of these compounds are not clear at the present time, although it has been reported that melatonin release was inhibited by lipoxygenase inhibitor. Since 22:6w3 is the major polyunsaturated fatty acid in the pineal body, its lipoxygenase products may be important mediators of the synthesis or release of melatonin.
Figure 1. Oxygenation of 22:6w3 by rat brain pineal body homogenate. Metabolites were separated on an Axxiom-ODS column (4.6 mm x 25 cm, 5 μ) using a gradient of a 0.1M ammonium acetate/methanol mixture changing from 40/60 to 15/85 in 50 min, then to 0/100 in 10 min, with a flow rate of 0.8 mL/min.

Figure 2. Production of oxygenated metabolites of 22:6w3 by porcine leukocyte 12-lipoxygenase.

13,14-epoxy-hydroxy-22:6w3 (m.w.=360)

Figure 3. Thermospray spectrum of the hepoxilin-like compound produced by porcine leukocyte 12-lipoxygenase.
Characterization of N-Linked Oligosaccharides by Electrospray and Tandem Mass Spectrometry

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INTRODUCTION

Many proteins of therapeutic value are glycoproteins, including antibodies, hormones, growth factors, clotting factors, immunomodulators, carrier proteins and a host of cell-attached receptors. Exploration of the role of complex sugar molecules in cell function could lead to a new class of carbohydrate-based therapeutics with a large potential market. Mass spectrometry, because of its sensitivity and selectivity, already has proven valuable as an analytical tool for characterizing oligosaccharides and glycoproteins [1-3]. Electrospray mass spectrometry promises to enhance the analytical capabilities of mass spectrometry toward the characterization of oligosaccharides and glycoproteins, by virtue of its great sensitivity and its ability to accurately measure the masses of large glycoproteins and resolve mass heterogeneities caused by differences in oligosaccharide structure. This paper presents electrospray mass spectrometric characterization of complex underivatized oligosaccharides and of ovalbumin, a glycoprotein with one occupied glycosylation site. Methodology developed for the mass analysis of oligosaccharide standards is applied to the mass analysis of a mixture of oligosaccharides that have been enzymatically digested from ovalbumin.

EXPERIMENTAL

Oligosaccharide standards were purchased from Dionex Corp. (Sunnyvale, CA). Ovalbumin was purchased from Sigma Chemical Co. (St. Louis, MO).

Enzymatic Digestion

10 mg of ovalbumin was dissolved in 200 mL of 200 mM sodium phosphate buffer containing 1% 2-mercaptoethanol. The sample was heated to boiling for 5 minutes and diluted to 1 mL with sodium phosphate buffer containing 0.6% NP-40. 20 milliunits of N-glycanase (Genzyme) was added to this mixture, and the enzymatic reaction was allowed to proceed for 12 hrs at 37 °C. An additional 20 milliunits of N-glycanase then was added and the mixture was incubated for another 24 hrs. The sample mixture then was diluted to 70% ethanol and incubated for 2 hrs at 4 °C. Precipitated protein was removed by centrifugation. Nonionic detergent was removed by application on two C-18 Sep-pak cartridges in succession, and salts and ionic detergent were removed by application on Dowex Ag-50W X-12 and QAE-sephadex in succession. The sample was evaporated under vacuum and 20% sent for electrospray mass analysis.

Mass Spectrometry

mass spectrometer: Sciex TAGA 6000E triple quadrupole with a mass range of 1400 amu.
sample concentration: 30 pmol/mL
sample solvent: water/methanol (4:1, v:v) containing either 10 mM ammonium acetate or 10 mM sodium acetate
infusion rate: 2 mL/min
scan range: 300-1400 amu for electrospray mass spectra
10-1400 amu for MS/MS spectra
scan rate: 100 amu/sec
spectral averaging: 5 scans- electrospray mass spectra
20 scans-MS/MS spectra
MS/MS collision gas: argon
MS/MS collision energy: 30-100 eV

RESULTS AND DISCUSSION

Electrospray mass spectrometry provided molecular weight assignments of oligosaccharides without prior derivatization. Oligosaccharides that were characterized in this study had a higher affinity for sodium or ammonium than for protons, so sample solutions were modified with sodium acetate or ammonium acetate to enhance the ionization efficiency of the oligosaccharides. Protonated oligosaccharides were difficult to generate when the sample solution was acidified and were only generated in high abundance when ammoniated oligosaccharides were subjected to
collision-induced dissociation to expel neutral ammonia. Singly-, doubly-, and triply-charged oligosaccharides were observed in the electrospray mass spectra resulting from the addition of one, two, or three cationic species to the oligosaccharides. Oligosaccharides that contain sialic acid did not exhibit a large ion current in the positive ion mode but did yield large ion signals in the negative ion mode. The maximum number of negative charges that were incorporated on these oligosaccharides (deprotonation) corresponded to the number of sialic acids contained on the oligosaccharide.

Collision-induced dissociation of oligosaccharides allowed differentiation of high mannose, complex, and hybrid oligosaccharides. Fragmentation generally occurred through dissociation of glycosidic bonds, and cleavage of the glycosidic bond adjacent to an N-acetylglucosamine was especially favored [4]. MS/MS analysis allowed identification of most of the structural sequence of the oligosaccharides, including the presence of core fucose units, but branch points were sometimes difficult to distinguish. Monomeric sugar units of the same mass and linkage positions could not be determined using this methodology.

Oligosaccharides present on the glycoprotein ovalbumin were enzymatically cleaved by N-glycanase and separated from the protein portion of the compound. Characterization of these oligosaccharides by electrospray and tandem mass spectrometry resulted in identification of two high mannose oligosaccharides (G2M6 and G2M5) as the most abundant sugars present on this glycoprotein. The relative abundances of digested ovalbumin oligosaccharides present in the electrospray mass spectrum were consistent with those obtained by ion chromatography/pulsed amperometric detection. However comparison of the carbohydrate content of ovalbumin as determined by both the electrospray mass spectrum of intact ovalbumin and the oligosaccharides digested (N-glycanase) from ovalbumin showed inconsistencies in the relative abundances. This was attributed to an incomplete digestion by N-glycanase, which favored enzymatic cleavage of lower molecular weight, high-mannose oligosaccharides.

**REFERENCES**


Use of Ammonium Acetate As A Matrix Additive: Enhancement of Oligosaccharide FAB Mass Spectra

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Fast atom bombardment mass spectrometry is a well-established method for the analysis of oligosaccharides and glycoconjugates. Recent work in our laboratory has focused on the use of matrix additives for the enhancement of native and derivatized oligosaccharide mass spectra. The results of investigating the utility of ammonium acetate as a matrix additive for the FABMS analysis of native and permethylated oligosaccharides are presented below.

The oligosaccharides studied were maltoheptaose (Sigma), isomaltoheptaose (BioCarb), OligoStandard GP-16 (Dionex), and OligoStandard GP-15 (Dionex). Permethylated derivatives were synthesized by reaction of the native sugars with methyl iodide/sodium hydroxide in dimethyl sulfoxide. Matrices used were: (1) GT-HCl: 1 ml each of glycerol (G) and thloglycerol (T) plus 5 drops of concentrated HCl, (2) GT-NH₄OAc: 1 mL each of G and T plus 10 drops of 1 M NH₄OAc, and (3) T-NH₄OAc: 2 mL T plus 10 drops of 1 M NH₄OAc. FABMS analyses were performed on a ZAB-SE double-focusing magnetic sector mass spectrometer (accelerating voltage 10 kV) equipped with a cesium ion gun (35 kV). Data was accumulated (5 scans) in the multi-channel analyzer (MCA) mode scanning over a 300-2000 amu mass range at 25 seconds per decade.

Maltoheptaose and isomaltoheptaose, 5 ug each, were analyzed by FABMS in the above three matrices. Protonated molecular ions MH⁺ were observed in all spectra. In the ammonium acetate matrices, MNH₄⁺ ions were also observed. The MNH₄⁺ species predominated over MH⁺ in T-NH₄OAc. No increase in ion intensity for the molecular ion species was observed in GT-NH₄OAc versus GT-HCl. However, in the T-NH₄OAc matrix, a 5-fold increase over GT-HCl in molecular ion intensity was observed. Although significant enhancement of molecular ion species for the native sugars was not observed in the ammonium acetate matrices, the overall signal/noise was improved due to a reduction in matrix background ions. A more dramatic effect was observed upon analysis of permethylated derivatives of maltoheptaose and isomaltoheptaose, 1 ug each, in the same three matrices. Increases of 15-fold and 50-fold were observed in GT-NH₄OAc and T-NH₄OAc, respectively, versus GT-HCl. The predominant ion in both GT-NH₄OAc and T-NH₄OAc was MNH₄⁺. Permethylated maltoheptaose was further analyzed at the 100 and 10 ng levels to determine the limit of detection. The 10 ng sample was easily detected with a signal/noise of 5:1 for MNH₄⁺. Shown in Fig. 1 are the spectra obtained for permethylated maltoheptaose at the 1 ug and 10 ng levels in T-NH₄OAc. Permethylated derivatives of two complex oligosaccharides, GP-16 (Fig. 2) and GP-15 (Fig. 3) were also analyzed by FABMS in the T-NH₄OAc matrix. Intense MNH₄⁺ ions and structurally significant fragment ions were observed. Finally, even though ammonium acetate has been demonstrated to be a useful matrix additive, one drawback that has been observed is the persistence of the additive in the ion source which necessitates more frequent source cleaning.

In summary, the addition of ammonium acetate to the FAB liquid matrix slightly enhanced the mass spectra of native oligosaccharides and significantly enhanced the mass spectra of permethylated oligosaccharides.
FIG. 1 MS ANALYSIS OF PERMETHYLATED MALTOHEPTAOSE

FIG. 2 MS ANALYSIS OF PERMETHYLATED GP-16

FIG. 3 MS ANALYSIS OF PERMETHYLATED GP-15

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DIFFERENTIATION OF TYPE 1 AND 2 CHAIN GLYCOSPHINGOLIPIDS
BY CID POSITIVE ION FAB-MS WITH B/E LINKED SCANNING
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CID/*FABMS spectra were obtained at constant B/E ratio from the pseudomolecular ions of
underivatized glycosphingolipids. The spectrum of a difucoglycolipid with type 1 chain core
structure, Leb ([M+H]+ at 1535.9 u.) is reproduced in Fig. 1A, while that of an isomeric type 2
chain structure, Lei ([M+H]+ at 1631.9 u.), is reproduced for comparison in Fig. 1B. As expected,
the spectra displayed a number of similar features. Ceramide ions, Z0, of Leb and Lei were found
at m/z 536 and 632, respectively. Ceramide containing ions, Y1, and Z1, and non-reducing end B1
ions were observed as shown in Schemes 1A,B. In both spectra the m/z 658 ion was observed in
high abundance, the result of preferential cleavage of the GlcNAc glycosidic bond (1) to form the
oxonium ion, (Fucor3-Gal[4-Fucor4]-3GlcNAc)*. Such preferential cleavage at HexNAc linkages
have been reported for both derivatized and native glycoconjugates in a variety of ionization modes
(1-4).

The primary ion m/z 658 further fragments via β-cleavages (3) to form a number of second
generation fragments, found at m/z 512, 366, 350, and 204. A number of pathways are possible,
as shown in Scheme 2. The spectra of Leb and Lei differ in the relative abundance of these product
ions. This clearly must depend on differences in rates of cleavage, which should in turn reflect
differences in the stereochemistry of the primary ions. For example, the m/z 350 ion is present
only in the spectrum of Leb and not observed in that of Lei. This could result from the influence
of the adjacent 2-NAc group. CID *FAB studies on synthetic model oligosaccharides, reported by
Laine et. al. (2), suggest that the instability of the 3-position of GlcNAc is due to steric crowding,
which restricts the distribution of energy among normal mode vibrations in the transition state for
glycosidic cleavage. In the vicinally branched Lewis structures, this restriction may be enhanced by
the presence of the residue linked in the adjacent 4-position, while in the case of the Leb isomer,
this effect may be further enhanced by the steric interaction between the NAc group and the
Fucβ1→2 residue on the type 1 linked Gal. The same phenomena may be responsible for the
appearance of fragments at m/z 1227 and 1209 in the spectrum of Leb, when no analogous
fragments were observed for Lei.

These differences were examined in more detail by further experiments employing CID daughter
ions from the primary ion m/z 658 (not shown), and precursor ion scans of the monosaccharide ion at m/z 204 (see Figure 2). In the daughter ion spectrum of m/z 658 from Lei,
production of some m/z 350 fragment could be observed. Nevertheless, the ratio of m/z 350/366
is clearly greater in the case of Leb. These results were further confirmed by precursor ion scans
for the m/z 204 fragment, which were obtained at much lower resolution, but which had the
advantage of eliminating contributions from the matrix ions. In the spectrum obtained from Lei
(Fig. 2B), the considerable abundance of the m/z 366 ion, compared to both m/z 350 and 512,
implies that m/z 204 is produced from the m/z 658 fragment chiefly by a sequence of three β-
eliminations: facile losses of the two Fuc residues from their respective sites, followed by loss of the
4-linked Gal residue. On the other hand, with Leb (Fig. 2A), a significant contribution is probably
made from loss of the 3-linked Fuc-Gal disaccharide as a unit to form the m/z 350 ion, followed
by loss of the 4-linked Fuc residue. Overall, the m/z 658 fragment appears to be somewhat more
stable in Lei, as evidenced by its greater abundance in the B/E spectrum.

Finally, it is worth noting a number of other common ions amongst the spectra shown in Figs.
1. The ion at m/z 264 may be produced by ring cleavage of the m/z 366 fragment (at bonds 2
and 5). The ions found at m/z 186, 168, and 138 have been proposed (2) to arise from further
successive decompositions of the m/z 204 fragment, representing (204-H2O), (186-H2O), and (168-
HCHO), respectively.

a. The nomenclature of Domon and Costello (5) has been adopted.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Figure 1. Spectra of A, type 1 chain, Lea, [M+H]+ at 285.5 u; and B, type 2 chain, Lea, [M+H]+ at 433.6 u. Marine 3-alkoxybenzyl Alcohol/crowe-5. Reduction of precursor ion by 50% with collision gas.

Figure 2. Proposed fragmentation pathways of A, type 1 chain, Lea, and B, type 2 chain. Lea, marine 3-alkoxybenzyl alcohol/crowe-5. Reduction of [M+H]+ by 50% with collision gas.

Scheme 1. Proposed fragmentation pathways of A, type 1 chain, Lea, and B, type 2 chain.

THE STRUCTURAL IDENTIFICATION OF EVERNINOMICIN-6

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Everninomics (D, B, C, 13-384-1 and 13-384-V), a novel class of oligosaccharides antibiotics, are produced by Microgonospora Carbonaceae. They are highly active against gram-positive bacteria and Neisseria, including strains resistant to beta-lactams, macrolides, lincomycins, tetracycline and rifampicin. Everninomics have many unusual structural features: an ortho ester functionality, an aliphatic methylene dioxy group, a completely substituted phenolic ester functionality and a nitro sugar. The structure elucidations of these Everninomics were carried out in our laboratories using chemical degradation, NMR and MS methods. Our subsequent studies using FAB and LSIMS indicated that these techniques are extremely useful for the structure analysis of this class of oligosaccharides.

This presentation describes the mass spectral analysis of a new Everninomicin component, EV-6, that further demonstrates that LSIMS (FAB) provides a simple, sensitive and structurally informative method for the analysis of this essentially non-volatile class of oligosaccharides. Both negative and positive ion LSIMS were highly successful in the identification of this new component. Negative ion LSIMS produced a strong (M-H)^- molecular ion and a single fragmentation series indicating the sugar sequence from the phenolic side of the molecule. Positive ion LSIMS did not produce a strong molecular ion, but the addition of NaCl to the EV-6 solution resulted in an intense (M-Na)^+ molecular ion. One also observed a more complex and far richer fragmentation pattern with NaCl enhanced positive ion LSIMS compared to negative ion LSIMS. Three distinct sugar sequence series were observed; one involving the phenolic side of the molecule and two involving the methylene dioxy side of the molecule. Thus, these complimentary LSIMS techniques provided both molecular ion information and fragmentation information that provided the sugar sequence.

REFERENCES


2b. B. N. Pramanik, P. R. Das and A. K. Bose, Journal of Natural Products, 1989, 52 No. 3, 534
QUANTIFICATION OF LEUKOTRIENE C4 AND N-ACETYL LEUKOTRIENE E4 IN RAT BILE USING CARBON-13 ISOTOPE DILUTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Cysteine-containing leukotrienes (LTs) are formed from arachidonic acid via the 5-lipoxygenase pathway and conjugation with glutathione, yielding LTC4. Sequential removal of the gamma-glutamyl and glycyl moieties yields LTD4 and LTE4, the latter of which may be further metabolized to N-acetyl LTE4. Leukotrienes are commonly quantified in biological extracts using a combination of HPLC and immunoassay. A strategy for GC-MS determination of cysteine-containing LTs has been reported by Murphy and coworkers (1,2). Hydrogenation and thioether cleavage yields 5-hydroxyeicosanoic acid as a common product of all the cysteine-containing LTs. Conversion to the pentafluorobenzyl (PFB) ester, trimethylsilyl (TMS) ether gives a derivative with excellent gas chromatographic properties that is detected with high sensitivity during negative ion electron capture MS. Application of the hydrogenation/derivatization/GC-MS approach has hitherto been limited by the lack of suitable internal standards; deuterium-labelled analogues are not suitable because of loss of the isotopic label during hydrogenation. We recently reported (3) the preparation of [13C4]-analogues of cysteine-containing leukotrienes and their use as internal standards is described here.

Abundant protonated molecular ions are generated during FAB MS of leukotrienes; recent work (4) has established that significant structural information is obtained by collisional activation of [M+H]^+ and [M-H]^− ions during tandem MS analyses. We have suggested (4) that FAB/tandem MS provides the basis for quantitative determination of cysteine-containing LTs and a preliminary report of this approach has been presented (5).

We report here the determination of cysteine-containing LTs in rat bile in an assessment of the role of these mediators in endotoxin shock. Aliquots (25 ul) of bile were supplemented with 400 pg of [13C4]LTC4 and 850 pg of N-acetyl [13C4]LTE4 and were separated by reverse-phase HPLC to give fractions corresponding to LTC4 and N-acetyl LTE4. These fractions were hydrogenated using a rhodium black catalyst. The products were converted to the PFB ester and further purified by straight-phase HPLC prior to preparation of the TMS ether derivative. During GC-MS analysis, ions of m/z 399 (corresponding to [M-PFB]^−) for the 5-hydroxyeicosanoic acid derivative) and m/z 403...
Biliary LT concentrations were determined in four animals prior to, and following, i.v. endotoxin administration. Marked increases in the concentrations of LTC₄ and N-acetyl LTE₄ were observed after endotoxin administration, with maximum values of approximately 250 pg/ul and 1500 pg/ul, respectively (Figures 1 and 2). Concentrations of LTC₄ were restored to pre-dosing levels 60 min after endotoxin but concentrations of N-acetyl LTE₄ remained elevated.

The production of cysteine-containing leukotrienes following endotoxin administration to the rat has been studied previously by Keppler and colleagues who postulated a role for these mediators in eliciting the cardiovascular effects characteristic of endotoxin shock (6). On the basis of HPLC isolations and RIA, these workers determined N-acetyl LTE₄ to be the principal cysteine-containing leukotriene present in rat bile. Estimated concentrations, however, were approximately 50 times lower than those determined in the present study. The discrepancy may be attributable to the different strain of rat used and/or to the lack of internal standardization in the immunoassay study.

References


Figure 1
Variation of concentrations of LTC₄ in rat bile with time in four animals. Bile duct cannulas were inserted at time zero and endotoxin was administered at 30 min. Leukotriene concentrations were determined using the GC-MS procedure.

Figure 2
Variation of concentrations of N-acetyl LTE₄ in rat bile with time in four animals. Bile duct cannulas were inserted at time zero and endotoxin was administered at 30 min. Leukotriene concentrations were determined using the GC-MS procedure.
A Method for the Study of Essential Fatty Acid Elongation and Desaturation Using Negative ion GC/MS.

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INTRODUCTION

Linoleic acid (18:2\text{co}6) is biosynthesized to arachidonic acid (20:4\text{co}6) via a series of enzymatic steps which carry out chain elongation and desaturation. Intermediates formed in the pathway to 20:4\text{co}6 are 18:3\text{co}6, 20:2\text{co}6, and 20:3\text{co}6. In addition, 20:4\text{co}6 is further transformed to minor amounts of 22:4\text{co}6 and 22:5\text{co}6. Desaturation occurs at specific sites along the carbon chain through the action of \Delta6 and \Delta5 desaturases which are membrane bound in the lumen of the endoplasmic reticulum.

A negative ion GC/MS method using pentafluorobenzyl derivatives (PFB) of the deuterated fatty acids was developed to investigate the in vitro biosynthesis of 20:4\text{co}6 from liver homogenates. This was compared to a method using methyl ester derivatives of the fatty acids in positive chemical ionization with the goal of decreasing detection limits and thereby lowering the amount of deuterated fatty acid needed in these analyses.

Incubation time, substrate concentration and cofactors were varied to examine their effects on product formation. All intermediates to d4-20:4\text{co}6 production were detected and quantified using 23:0 as an internal standard. The d4-20:4\text{co}6 was not fully resolved from the d0-20:4\text{co}6. Isotopic ratios were used to determine the quantity of d4-20:4\text{co}6 formed during the incubation.

METHODOLOGY:

LIVER PREPARATION-INCUBATION

Homogenates of whole rat liver were prepared according to the method of Cook (1). Adult male rats (Sprague-Dawley) were fed a standard laboratory chow diet and decapitated. Livers were removed and the tissue was homogenized in glass tubes with 0.1 M-phosphohate buffer at pH 7.4 containing 2 mM reduced glutathione and 25 mM sucrose (4 ml medium per g tissue). Protein content of fractions were determined by the method of Lowry, et. al.(2) Incubation mixtures contained 0.1 M-phosphate buffer at pH 7.4, 0.2 mM-Co-A, 2 mM-MgCl2, 2 mM-ATP, 0.5 mM-NADH, 0.5 mM-NADPH, 0.5 mM malonyl-CoA, 0.025 mM fatty acid and 0.050 mM BSA. Protein 4-7 mg was added to the incubation mixture in a final volume of 2 ml. Samples were incubated at 37° C for 15 min in a shaking water bath.

EXTRACTION-DERIVATIZATION

Reactions were stopped by the addition of 4 ml of 10% methanolic KOH. The fatty acid methyl ester, 23:0, (5 ug) was added as an internal standard. Mixtures were heated at 65° C for 30 min and acidified with 7N-HCl. Samples were extracted with 50% diethyl ether in petroleum ether 3X, dried over anhydrous ammonium sulfate, divided into two fractions and evaporated under N2. One fraction was derivatized to the fatty acid methyl esters with BF3 in methanol (14% w/v). Samples were brought up in 1 ml heptane. The other fraction was derivatized to the PFB esters with 70 ul of a solution of pentafluorobenzyl bromide, disopropylamine in acetonitrile (1:10:1000) and heated at 60 deg. for 15 min. The solvent was evaporated and the residue dissolve in 1 ml heptane for GC/MS analysis.

GC/MS CONDITIONS

Fatty acid methyl esters (ME) and PFB esters were run on a 25 \text{um} coated, 30 m x 0.25 mm i.d., DB-FFAP capillary column using splitless injection. The oven temperature was programmed from 80°-185°@20°/min, 185°-240°@10°/min and held for 30 min. The injector temperature was 250° and transfer line 280°. Source temperature was 150°. The source pressure of methane (99.99%) was 4 x 10^-4 torr. Tally acids were analyzed in the selected ion mode using the M+1 ion for the ME derivatives and the M-PFB ion for the PFB derivatives. Source tuning and system optimization was carried out for each ionization mode and the same electron multiplier voltages were used for both positive and negative.
CONCLUSION

A negative ion GC-MS method was used to follow the course of conversion of d4-18:2ω6 acid to d4-20:4ω6 in in vitro preparations of rat liver homogenates. Intermediates in the biosynthesis of d4-20:4ω6 were characterized. Incubation conditions such as protein concentration, cofactors, and time were varied to evaluate optimum product formation. Fatty acids were derivatized to their PFB esters and the M-PFB ion was observed in the negative ion mode. The deuterated fatty acids eluted just prior to the unlabeled counterparts. The d4-20:4ω6 fatty acid co-eluted with the unlabeled material.

Deuterated fatty acids were quantified using a standard curve, generated from the ion ratios of the M-PFB fragments of the internal standard, 23:0, and the analyte against a known concentration of the internal standard.

This method was compared to the detection of the M+1 ions of methyl esters of deuterated compounds in the positive ion mode. Negative ion detection was generally three orders of magnitude more sensitive than positive ion detection. Ten femtograms of the pure d4-18:2ω6 PFB ester was detected with a signal to noise ratio of 3 in the negative ion analysis. Fatty acids labeled with four or more deuterium atoms were preferred because of the diminished ion interference resulting from the M+4 isotope of the unlabeled compound. This method makes possible cost effective metabolism studies because smaller quantities of the deuterated fatty acid are needed.

REFERENCES


Nutritional Effects on In vitro Prostaglandin Production In Rat Brain Slices
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ABSTRACT
In order to assess the role that alcohol exposure together with dietary lipid manipulations have on the capacity of the brain to produce prostaglandins (PGs), in vitro, rats were placed on diets where the lipid content was precisely controlled. Animals were maintained on 10% fat diets supplemented with 9% of either borage (23% 18:3ω6), olive (72% 18:1ω9), or menhaden fish (14.7% 20:5ω3 and 8% 22:6ω3) oils. After 2 weeks of dietary treatment ethanol was administered to the animals for a period of seven days by inhalation. Control and treated animals were then decapitated, whole brains were sectioned into slices and incubated at 37° with the calcium ionophore A23187 and CaCl2. The supernatants were collected, prostaglandins extracted, and PGD2, PGE2-α, 6-keto-PGF1-α and TXB2 were derivatized to their pentafluorobenzyl-trimethylsilyl-methoxime derivatives and analyzed by negative ion chemical ionization by gas chromatography/mass spectrometry.

INTRODUCTION:
Dietary unsaturated fats provide a major component of the fatty acids which are incorporated into membrane phospholipids. Animals elaborate dietary linoleic (18:2ω6) and linolenic (18:3ω3) acids to the chain elongated highly unsaturated fatty acids (HUFAs). The fatty acid, 18:2ω6 is converted to 20:4ω6 (arachidonic acid) and 18:3ω3 to 22:6ω3 (docosahexaenoic acid) via a series of shared desaturating and elongating enzymes which reside in the lumen of the smooth ER of hepatocytes. The ratio of dietary 18:2ω6 to 18:3ω3 is important in determining the final composition of membrane phospholipids which are the substrates for the oxygenated derivatives of fatty acids, the prostaglandins, leukotrienes, and lipoxins. Phospholipase A2 (PLA2) releases HUFAs to the cytosol where they are converted to prostaglandins, thromboxanes and prostacyclin via the cyclooxygenase pathway. 20:4ω6 is the substrate for the two series prostaglandins. Prostaglandins carry out a number of regulatory roles in homeostasis. PGD2 regulates body temperature and sleep. PGE2-α is a smooth muscle contractor. PGI2 increases vasodilation and inhibits platelet aggregation. Thromboxane A2 (TXA2) increases platelet aggregation and vasoconstriction. Ethanol has been shown to ameliorate PLA2 activity and thereby effect substrate levels for prostaglandin production.

EXPERIMENTAL
Rats were fed three diets consisting of the nutrients specified in the AIN-76A diet but varied in lipid composition for 21 days. The lipid content was varied by adding 9% of either borage oil (23% 18:3ω6) olive (78% 18:1ω9) or fish (14.7% 20:5ω3 8.7% 22:6ω3) plus 1% corn oil (78% 18:2ω6). Alcohol was administered by inhalation beginning the third week. Animals were decapitated after the seventh day, and the entire brain removed. One half of the cerebral hemisphere was sliced into approximately 100 mg slices and assayed for cyclooxygenase activity.

Tissue slices were rinsed twice with 1 ml cold PBS (pH 7.4). Samples were placed in 1.5 ml of PBS with 5% glucose. Indomethacin controls were pre-incubated with the 10 μM of the reagent. At zero time, 20 μl of 2 mM CaCl2 and 10 μM of the calcium ionophore, A23187 were added to the slices. 400 μl of the supernatant was removed for zero time point comparisons. Samples were incubated for 30 min at 37° in a shaking water bath. The reactions were stopped by the addition of 60 μl of formic acid.

The pH was adjusted to approximately 6.5 by adding 100 μl of pyridine. 800 μl of a 2% solution of methoxime HCl was added to each sample. Samples were mixed on a vortex and allowed to stand overnight at room temperature. The methoxime derivatives were extracted on SEP-PAK C18 cartridges with 2 ml methanol after conditioning the columns. The solvent was evaporated under nitrogen and samples were reacted for 30 min at 60° with pentafluorobenzyl bromide and dimethoxypropylamine in acetonitrile (1:10:1000) to their PFB esters. Reagents were evaporated under nitrogen and trimethylsilyl ethers were formed by adding 50 μl of BSTFA and 50 μl of acetonitrile at 60° for 15 min. The reagents were evaporated and the samples brought up in 40 μl of dodecane.

Samples were analyzed in the negative ion mode on a Kratos MS80 mass spectrometer with a 15 m X 0.25 mm DB5 capillary column using on-column injection. The oven temperature was programed...
from 215 to 310 at 30° per min. Methane, 0.5 torr was the chemical ionization gas and the M-PFB ions of the prostaglandin derivatives were detected. The prostaglandins were quantified using tetradeuterated internal standards added to the samples prior to derivatization. Thromboxane (TXB2) was quantified using the ion from d4-6-keto-F1-α with a response factor of 0.71. Final calculations were based on 100 mg of tissue, wet weight. Prostaglandins from Indomethecin and zero time controls were subtracted from quantities formed after 30 min incubation.

RESULTS

PGD2. Tissue from control animals on fish oil diets had significantly lower levels of PGD2 than tissue from olive oil fed animals. No significant differences were found between ethanol treated and control animals.

PGF2α. Tissue from animals receiving olive oil in their diet produced significantly higher levels of PGF2α than tissue from either two control groups. Significantly lower levels of PGF2α formation were found in ethanol and control animal comparisons from the olive and fish oil groups.

6-Keto-PGF1α. Tissue from animals receiving fish oil produced significantly lower amounts of 6-keto-PGF1α than tissue from animals on the olive diet. The ethanol exposure had opposite effects in the borage and olive oil diets that were statistically different from their controls.

TXB2. Tissue from animals on the fish oil diet produced significantly lower levels of Thromboxane TXA2 (it's metabolite TXB2 is actually measured) than tissue from the olive oil group.
IN VITRO Desaturation of Deuterated Linoleic Acid (d₄-18:2ω6) to d₄-18:3ω6 In Adult Rat Livers from Alcohol Exposed Animals.

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INTRODUCTION
Desaturation of the essential fatty acid, linoleic acid (18:2ω6) to 18:3ω6 occurs through the action of membrane bound Δ6 desaturase within the endoplasmic reticulum. Dietary lipids are known to influence the fatty acid composition of membranes in various tissues. Chronic alcohol exposure has a profound affect on fatty acid metabolism within the liver. In this study, rats were maintained on normal diets supplemented with either fish oil or corn oil as a lipid source and then exposed to alcohol vapors for seven days. The animals were killed and liver homogenates assayed for Δ6 desaturase activity using deuterated linoleic acid (d₄-18:2ω6). Fatty acids were derivatized to their pentafluorobenzyl esters and analyzed by NCI GC/MS. In addition, 18:2ω6 and 20:4ω6 were measured in the tissue and the amount of 18:2ω6 was correlated with enzyme activity.

EXPERIMENTAL
Adult male Sprague-Dawley rats (control groups n=3, ethanol exposed groups n=4) were maintained on a rat chow diet for two weeks that was supplemented with either corn or fish oil as a primary source of lipid. Ethanol treated animals were then placed in alcohol inhalation chambers for 7 days and continued on their same diets. On the seventh day animals were killed and the livers removed, homogenized in PBS buffer (pH 7.4) with 2mM glutathione. Liver homogenates were incubated with 25 μM d₄-18:2ω6 complexed to BSA 50 μM and containing 0.2 mM CoASH, 2 mM MgCl₂, 2 mM ATP, and 0.5 mM NADH at 37°. for 15 min. Reactions were stopped with addition of 4 ml 10% KOH in methanol and heated to 85° for 1 hour. Samples were acidified with 14 N HCL and extracted with ether:hexane 1:1 three times and derivatized to their PFB esters with pentafluorobenzylbromide, diisopropylamine in acetonitrile for 15 min at 60°. The reagent was evaporated under nitrogen and samples brought up with 1 ml heptane and injected onto a 30 m X 0.25 um FFAP column and analyzed in the selected ion mode by negative ion GC/MS.

FINDINGS
Liver concentrations of 18:2ω6
Significantly higher amounts of 18:2ω6 were found in livers from rats on the corn oil diet than in livers of those animals on the fish oil diet (p< .02). The ethanol groups also had higher levels of 18:2ω6 than the controls and this was significant at p< .05 in animals on the corn oil diet

Liver concentrations of 20:4ω6
Fish oil fed animals from control groups had lower levels of liver 20:4ω6 than livers from corn oil fed animals. However, no significant differences in levels of 20:4ω6 were found in ethanol treated animals on either diet.

Ratio of 20:4ω6 to 18:2ω6 In livers
The ratio of 20:4ω6 to 18:2ω6 appeared about equal in control animals on either diet. Ethanol treated corn oil fed animals had a lower ratio of 20:4ω6 to 18:2ω6 than did control animals. This difference was due to the higher levels of 18:2ω6. The ethanol treated fish oil group had a lower 20:4ω6 to 18:2ω6 ratio but this was not significantly different from the control group.

Δ6 desaturation in corn and fish oil fed animals.
Desaturation of d₄-18:2ω6 to d₄-18:3ω6 was highest in livers from those animals on the fish oil diet. Ethanol treated animals on either diet had lower levels of enzyme activity than their controls. Fish oil fed animals with and without ethanol had higher levels of enzyme activity than corn oil fed animals.
DISCUSSION:
Dietary lipids effect fatty acid liver composition and metabolism. Animals fed a corn oil based diet (52% 18:2ω6) had an overall higher level of 18:2ω6 in liver tissue than those on the fish oil diet (1.5% 18:2ω6). The same animals also had higher levels of 20:4ω6 in the liver than fish oil control group. Desaturation of d4-18:2ω6 was lowest in the corn oil group and highest in the fish oil group. This data suggests that the desaturase may be regulated by end-product inhibition. An alternative explanation fitting this data would be substrate inhibition. Similar amounts of 18:2ω6 were found in fish oil ethanol and corn oil control groups. These two groups also had similar desaturase activity.

Ethanol increased the liver concentration of 18:2ω6. Although the mechanism for this is not known, it may be due to cofactor competition by alcohol dehydrogenases (ADH) and the desaturase. The oxidation of ethanol to acetaldehyde by the microsomal ethanol oxidizing system (MEOS) ADH requires NADPH which is also needed in the fatty acid chain elongation step. Ethanol’s oxidation may possibly explain increased levels of 18:2ω6 in liver. It is reasonable to assume from this data that alcohol plays some role in essential fatty acid metabolism whereby it leads to elevated levels of the fatty acid precursor. In addition, these elevated levels were negatively correlated with desaturase activity. Diets high in 18:2ω6 had higher levels of this fatty acid in liver tissue with comparatively lower desaturase activity. Conversely diets low in this substrate such as fish oil had higher enzyme activity.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Characterization of 14,15-Epoxyeicosatrienoid Acid Metabolites from Human Platelets by GC/MS.

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The epoxyeicosatrienoic acids (EETs) are cytochrome P-450 mediated oxidation products of arachidonic acid (AA) (1). Exogenous EETs have been reported to influence the function of endocrine, vascular and renal tissues, suggesting a possible mediator role for these compounds. It has also been proposed the human platelet can synthesize the EETs (2). We have shown that EET formation by human platelets is simply due to autooxidation of AA. Although the EETs are not produced from the platelet there is still potential for interaction with the platelet enzyme systems with EETs released from vascular tissue.

Human platelets were obtained from Vanderbilt Clinical Blood Bank. The platelets were centrifuged at 500 x g for 15 min. The pellets were collected and suspended in buffer (145 mM NaCl, 5 mM KCl, 5.5 mM Dextrose, 1 mM MgCl2, 0.2 mg/ml BSA, 100 mM HEPES, pH 7.4) and adjusted to a suspension of 1 x 10^9 platelets/ml. Aliquots of platelet suspensions (5 ml) were incubated with 14,15-EET (5 mCi/mmol, 3 uM) at 37 °C for different time (5 min to 1 hr). The incubations were stopped by addition of 5 ml of iced-cold methanol. The whole lipids were extracted into chloroform/methanol (2/1, V/V). The extracts were separated by TLC or reversed phase HPLC. The phospholipids fractions were isolated and then separated by normal phase HPLC. The metabolites were isolated and converted to the corresponding methyl ester (ME) and pentafluorobenzyl ester (PFB). The derivatives were further purified by HPLC and converted to their trimethylsilyl (TMS) derivatives for GC/MS analysis.

EI and GC/PCI-MS were performed on a Finnigan-MAT INCON 50 quadrupole mass spectrometer interfaced with Hewlett Packard 5890 gas chromatograph. GC/NICI-MS was carried out on a Nermag R-1010C quadrupole instrument equipped with a Varian Vista 6000 gas chromatograph. Chromatography was carried out a SPB-1 (15 m x 0.32 mm ID., Supelco) fused silica capillary column. The temperature of injector, transfer line and ion source was kept at 260 °C. Helium and methane were used as a carrier and regent gas, respectively. The GC oven temperature was held at 190 °C for 0.5 min, then programmed to 320 °C at 20 °C/min, then held for 5 min at this temperature.

When 14,15-EET was incubated with human platelets, it was metabolized to four compounds. The EI mass spectrum of metabolite 1 as its methyl ester (ME), trimethylsilyl (TMS) derivative showed a base peak at m/z 295, suggesting a hydroxy group at C-12 (Figure 1). The NICI mass spectrum of its pentafluorobenzyl (PFB), TMS derivative was dominated by the ion of m/z 407 corresponding to (M-PFB). These data suggested that metabolite 1 was 12-OH-14,15-EET. The EI mass spectrum of the ME-TMS derivative of metabolite 2 exhibited significant fragment ions at m/z 173, 275, 323 indicating two hydroxy groups at C-14 and C-15. The NICI mass spectrum of its PFB, TMS derivative gave a base peak at 481 (M-PFB). This suggested that metabolite 2 was 14,15-dihydroxyeicosatrienoic acid (DHET). Similarly, the EI mass spectrum of metabolite 3 as its ME-TMS derivative showed characteristic ions at m/z 295, 173, 275, indicating the presence of three hydroxy groups at C-12, C-14, and C-15. Its PCI spectrum exhibited a protonated molecular ion at m/z 585 and subsequent elimination of three TMSOH from the protonated molecular ion. This resulted in the formation of ions at m/z 495 (MH-TMSOH), 405 (MH-2TMSOH) and 315 (MH-3TMSOH). In addition, an intense ion at m/z 669 (M-PFB) appeared in the NICI mass spectrum of its PFB-TMS derivative. Thus, metabolite 3 was characterized as 12,14,15-trihydroxyeicosatrienoic acid (THET). Metabolite 4 was identified as phosphatidylcholine containing 14,15-EET at C-2 by comparing its retention time on HPLC with that of authentic compound. 12,14,15-THET was formed from 12-OH-14,15-EET via epoxide hydrolase. 14,15-DHET was not a substrate for 12-lipoxygenase.

In summary, human platelets metabolized 14,15-EET into the corresponding 14,15-DHET, 12-OH-14,15-EET and 12,14,15-THET by epoxide hydrolase and 12-lipoxygenase. Also, 14,15-EET was mainly incorporated into phosphatidylcholine at C-2 position of glycerol (Figure 2).
References:

Acknowledgement:
This work was supported by NIH grant DK 38226.

Figure 1, EI mass spectrum of 12-OH-14,16-EET ME-TMS derivative

Figure 2, Proposed Biotransformation Routes of 14,15-EET in Human Platelets

INTRODUCTION: Eicosanoids are important endogenous mediators in the pathology of respiratory diseases, making measurement of their relative concentrations following various treatments important in further elucidating their role. While GC/MS has become the definitive methodology for detection of the isolated eicosanoids, their sampling remains a challenge. Measurement of the eicosanoids from plasma has been questioned by many workers because of sampling artifacts, while measurement of metabolites in the urine can be difficult to relate back to respiratory production. A novel methodology for sampling the large airways utilizing microdialysis may provide an in-vivo means of observing post-treatment changes in eicosanoid production.

METHODS: Microdialysis probes were of radial design, constructed of nested fused silica tubing and 4 mm of exposed dialysis membrane having a molecular weight cutoff of 40,000 dalton. The probe was perfused at 3.8 µL/min with PBS solution. The in vitro recoveries were determined immediately prior to use using standard solutions. Male Hartley guinea pigs (400-500 g) were presensitized two weeks prior to experiments with an i.p. injection of ovalbumin and Al(OH)3. At the start of an experiment, guinea pigs were anesthetized by an i.p. injection of urethane, supplementary glucose supplied was i.v., and a tracheotomy was performed. Microdialysis probes were inserted approximately 2 cm into the tracheal opening past a guide cannula. Samples were collected into 4 mL polypropylene tubes for six one hour intervals. The treatment groups received either saline or ovalbumin i.v. during the second hour of microdialysis sampling. Prostaglandins E2, D2, F2α and 6-keto-F1α and thromboxane B2 were determined by GC/MS. Tetradeuterated standards were added to aqueous solutions, which were treated (1% MOX-HCl, pH 5-7) overnight to produce the N-methoxime, extracted with C18 SPE, derivatized to the PFB ester (0.1% PFBBr, 60°C for 15 min), TMS ether (BSTFA,60°C for 15 min), and dissolved in 50 µL dodecane. Derivatives were injected on-column into a 1 m retention gap and separated in less than 3 min on a 15 m, 0.25 mm i.d. DB-5 column housed in an HP5890 gas chromatograph. The HP5988A mass spectrometer was operated in resonance electron-capture ionization mode, with SIM switching between the predominant [M-PFB]- ion for each eicosanoid.

RESULTS: Microdialysis probe recoveries varied between 5 and 10%. Intraday variations for a given probe were less than 1% and interday variations, including before and after an animal experiment, were less than 2%. The eicosanoids quantified in the present study were separated in under 3 minutes (Figure 1 displays an exemplary separation). PGE2, PGD2, PGF2α were quantified in most microdialysis samples. TXB2 showed inconsistent assay recovery leading to large day-to-day variations, while 6-keto-PGF1α was below current quantification limits in most samples. In control guinea pig experiments, in which animals were unsensitized and unchallenged, consistent eicosanoid levels below 200 pg/sample were observed. For sensitized guinea pigs, treated with saline only, PGE2, PGD2, PGF2α levels tended to increase in the final three hours of sampling. Ovalbumin treatment did not produce a large increase or decrease in any of the measured eicosanoids, however, levels of PGE2, PGD2, PGF2α and TXB2 tended to increase in first hour following ovalbumin challenge. Table 1 summarizes levels for each group with all time points averaged, while Figure 2 shows the time resolved means for PGD2.

DISCUSSION: Eicosanoids have been successfully measured in the trachea of anesthetized guinea pigs. In particular, mean levels for PGE2, PGD2, PGF2α and TXB2 were in the range 20-60 pg / one hour microdialysis sample. Levels for 6-keto-PGF1α were typically below the standard curve's lowest point (10pg/sample). No statistically significant increases or decreases were observed upon challenge with ovalbumin, however, every animal responded with some increase in PGE2, PGD2, PGF2α and TXB2 in the first hour following challenge. Clearly, a more robust change will be necessary to observe a statistical increase. In conclusion, in-vivo microdialysis has been demonstrated to be a potentially useful means of sampling the trachea as a measure of respiratory production of eicosanoids. Future experiments will address the possibility of concurrent measurement of respiratory function, as well as the effect of more specific challenge protocols (e.g. LTD4 challenge).

<table>
<thead>
<tr>
<th>Eicosanoid</th>
<th>Control</th>
<th>Saline</th>
<th>Ovalbumin</th>
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<tr>
<td>PGE2</td>
<td>31.168 ± 4.785</td>
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<tr>
<td>6-keto-PGF1α</td>
<td>7.702 ± 2.612</td>
<td>3.120 ± 0.792</td>
<td>4.470 ± 1.048</td>
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EICOSANOID LEVELS IN GUINEA PIG TRACHEA MICRODIALYSIS SAMPLES (Mean ± SEM, all time points)
Figure 1: GC/MS chromatogram of eicosanoids sampled in the 6th hour of guinea pig tracheal microdialysis

Figure 2: Levels of PGD$_2$ (pg/sample, mean ± SEM) in tracheal microdialysis samples (6 one hour samples) from control, saline treated, and ovalbumin treated guinea pigs.
SIMULTANEOUS GC-MS QUANTITATION OF PLATELET ACTIVATING FACTOR (PAF) AND 1-0-HEXADECYL-2-LYSO-SN-GLYCERO-3-PHOSPHOLIPIDS IN BRONCHOALVEOLAR LAVAGE (BAL) FLUID FROM HUMAN LUNG.


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PAF is a phospholipid mediator released by inflammatory cells in vitro in response to antigenic and nonantigenic stimuli. Demonstrated biological activities of PAF suggest that PAF released by inflammatory cells in human airways in vivo may modulate, in part, certain characteristic events in asthma such as cellular infiltration, microvascular leakage and bronchial hyperreactivity. Assessment of in vivo biosynthesis of PAF in the airways of a human model of asthma is therefore, critical for determination of the role of PAF in asthma. If significant metabolism of PAF does not occur in airway fluid, a reliable index of in vivo PAF production in peripheral airways of asthmatic subjects may be obtained via measurement of levels of the phospholipid mediator in BAL fluid obtained via a standardized wedged bronchoscope technique. Studies of the metabolism of PAF to its 2-lyso-metabolite (lyso-PAF) in BAL fluid in vitro indicate that the rate of PAF deacylation in BAL fluid varies significantly depending upon whether an antigenic response has been initiated. The life-half of PAF is > 90 minutes in BAL fluid from unchallenged subjects and < 12 minutes in BAL fluid from antigen-challenged subjects. These findings indicate that assessment of PAF levels in BAL fluid may not always provide a reliable index of in vivo PAF biosynthesis in airways of human asthmatic subjects, particularly in BAL fluid from subjects after antigen challenge.

Since measurement of PAF levels in BAL may not provide a reliable index of in vivo production of the phospholipid mediator in human airways, simultaneous measurement of PAF and lyso-PAF in BAL may provide an index of in vivo production of PAF in human airways. Aliquots (2 ml) of BAL fluid were first treated immediately with 8.8% formic acid (20 μl/ml BAL fluid) for inactivation of acid-labile acetylhydrolase. Two to 10 ng of 2H3-PAF and 2H4-lyso-PAF with 2H-label in the 1-0-hexadecyl moiety were added to 2 ml aliquots of acidified BAL fluid as internal standards. The samples were submitted sequentially to protein denaturaton (40% methanol; v/v), solid phase extraction (C18), elution with methanol, drying under N2, phosphate ester hydrolysis with 49% HF, hexane extraction of HF hydrolysis products, drying under N2, and pentafluorobenzoyl esterification of the hexane-extracted residue. Following hexane extraction of pentafluorobenzoyl esterification products, 0.5-1.5 μl aliquots of the residue containing the analytes and 2H-analogs of PAF and lyso-PAF internal standards dissolved in 30 μl dodecane were subjected to GC-MS analysis. Molecular anions at m/z 552, 555, 704 and 708 were simultaneously monitored for detection of injected analytes and internal standards as shown in Figure 1. Signal at m/z 552 and 555, respectively, are characteristic for the pentafluorobenzoyl ester of the HF hydrolysis product of PAF and 2H4-PAF (Panel A). Ions at m/z 704 and 708, respectively, represent signal from molecular anions of bis(pentafluoro-benzoyl) esters of HF-treated 1-o-hexadecyl-2-lysoglycerophospholipids (lyso-C16-alkyl-PL) and 2H4-lyso-PAF (Panel B). Simultaneous GC-MS analysis of PAF and lyso-C16-alkyl-PL extracted from BAL fluid is completed within < 30 minutes and are of adequate purity for detection of low picogram levels of analytes. Standard curves relating ratios of PAF/2H3-PAF and lyso-PAF/2H4-lyso PAF added to BAL fluid are linear from low picogram to nanogram ranges with < 20% inter- and intra-assay variations.

Levels of PAF and 1-o-hexadecyl-2-lysoglycerophospholipids extracted from BAL fluid from an asthmatic subject are shown in Figure 2. PAF levels in BAL fluid obtained at the different time intervals (30 minutes and 24 hours after antigen) do not vary significantly indicating either that significant in vivo PAF biosynthesis does not occur in the airways or that PAF deacylation occurs with such rapidity that the phospholipid mediator may not be consistently present in BAL fluid.

Elevations in lyso-C16-alkyl-PL in BAL fluid reflect accumulation of this family of phospholipids in human airways in vivo. Potential sources of the lyso-C16-alkyl-PL in BAL fluid obtained subsequent to antigen challenge include deacylation of PAF, phospholipase A2 catalyzed hydrolysis of fatty acids from 1-o-alkyl-2-acyl-sn-glycero-3-phospholipids. Further studies are required for determination of source of lyso-C16-alkyl-PL in BAL fluid observed subsequent to antigen challenge and the portion of these compounds derived from PAF deacylation.
Figure 1: Detection of PAF and 1-O-hexadecyl-2-lyso-sn-glycero-3-phospholipids extracted from BAL fluid. Ordinate = signal intensity. Abscissa = time in minutes: seconds.

Figure 2: Endogenous levels of PAF and 1-O-hexadecyl-2-lyso-sn-glycerophospholipids in human BAL fluid 30 minutes and 24 hours after instillation of normal saline and antigen. PAF levels = pg/ml. 1-O-hexadecyl-2-lyso-sn-3-glycerophospholipids = ng/ml.
MAKING THE TRANSITION FROM HPLC TO GAS PHASE ANALYSIS IN STRUCTURAL STUDIES OF ARACHIDONIC ACID METABOLITES

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Mass spectrometry has played a singular role in the structure elucidation of the ever-increasing members of the arachidonic acid cascade family including leukotrienes, prostaglandins and cytochrome P450 metabolites. Electron ionization GC/MS analysis of derivatized eicosanoids provides critical structural information on carbon skeleton modifications, while molecular weight information is obtained by positive ion CI or negative ion electron capture GC/MS analysis of pentafluorobenzyl ester derivatives. These strategies combine the high specificity of mass spectral analysis with the high selectivity of capillary column gas chromatography.

Problem: Biological extracts require exhaustive purification

Arachidonate metabolites thermally unstable

Solution: HPLC purification- metabolites found by radioactivity detection

Negative Ion Electron Capture LC-MS

Thermospray Vaporizer
(No Electrolytes)

Discharge Electrode

High Sensitivity LC-MS

Molecular Weight Information

High Sensitivity LC-MS

METABOLISM OF 12(R)-HETE. 12(R)-hydroxy-5,8,10,14-eicosatetraenoic acid [12(R)-HETE] is one of the major cytochrome P450-dependent arachidonate metabolites in the bovine, rabbit and human corneal epithelial microsomes. 12(R)-HETE is biologically active. In addition to chemotactic and chemokinetic properties, this eicosanoid inhibits Na-K ATPase activity in corneal tissues.

We also reported that 12(R)-hydroxy-5,8,10,14-eicosatetraenoic acid is formed from arachidonic acid in corneal epithelial microsomes and has unique biological activities also, including vasodilatation and neovascularization. However the synthetic pathway of this compound was not fully elucidated. It was postulated that this dihydro compound is formed from arachidonic acid via either an 11,12-epoxide intermediate by epoxide rearrangement and keto reduction or via 12(R)-HETE by an oxidation and two consecutive keto-reduction steps. We, therefore, studied the metabolic degradation of 12(R)-HETE in corneal epithelium, the tissue of origin, and identified the major metabolites in intact and broken cell preparations.

Possible Pathways in the Formation of 12(R)-HETE

Arachidonic Acid

\( \text{COOH} \)

OH 12(R)-HETE 11,12-EET

\( \text{COOH} \)

\( \text{O} \) 12-oxo-ETE 12-oxo-ETrE

\( \text{OH} \) 12(R)-HETE

These studies were done in collaboration with Dr. Michal Lanilo Schwartzman at the New York Medical College in Valhalla, NY. Tissue preparation, 12(R)-HETE incubations and initial purification (extraction and first HPLC separation) were all performed in Dr. Schwartzman’s laboratory. Samples were then sent to National Jewish Center for analysis.

HPLC-Mass Spectrometry. HPLC-MS was performed on a Vestec model 201 quadrupole mass spectrometer with thermospray Interface (Vestec Corporation, Houston, TX). Samples were analyzed by negative ion electron capture mass spectrometry following conversion of carboxyl moieties to the pentafluorobenzyl esters. Samples were taken to dryness then reacted with 10 ul of pentafluorobenzyl bromide and 10 ul of n,n-disopropylethylamine in 100 ul acetonitrile for 30 minutes at room temperature. Excess reagent was removed under reduced pressure and the PFB-esters were separated by reverse-phase HPLC on a Phenomenex 5 u C18 ODS column (250 X 4.6 mm, Jones Chromatography, Columbus, OH) using a linear gradient from 60% acetonitrile:water to 100% acetonitrile in 15 min and then isocratic for 30 min in 100% acetonitrile. Fractions were collected and appearance of
radioactivity determined by scintillation counting techniques. Radioactive fractions from the HPLC were introduced into the mass spectrometer through the thermospray interface with the control tip temperature at 200 °C and the thermospray source at 300 °C.

**IDENTIFICATION OF PEAK V.** Following derivatization as the pentafluorobenzyl ester, HPLC chromatography separated many components; however the radioactivity was detected in two fractions, fraction 28 (45%) and fraction 29 (29%). At this retention time there were no compounds eluting which had UV absorption at 236 nm, a chromophore diagnostic for conjugated dienes. LC-MS analysis of the component eluting at this retention time (28-29 min) revealed production of abundant ions at m/z 319 and 321. Considering that m/z 319 was identical to that of the starting carboxylate anion from 12(R)-HETE and yet this component lacked substantial UV absorbance at 236 nm, this metabolite was likely isomeric, for example a 12-oxo-metabolite. With the possible existence of a keto moiety in this metabolite, the methoxylamine derivative was made prior to trimethylsilylation in order to improve gas chromatographic behavior for GC-MS analysis. By negative ion GC-MS this metabolite formed two derivatives (ECL=21.08 and 21.18) with very similar spectra containing abundant ions at 348 (M-181) and m/z 206 (loss of 111 and CH3O from m/z 348). This later ion would be expected for the methoxime derivative following cleavage of the carbon bond adjacent to the imino moiety. The two separable components would correspond to the syn and anti-isomers typically observed with methoxime derivatives. Furthermore Peak V was also found to co-elute with synthetic 12-oxo-5,8,14-eicosatrienoic acid on HPLC. All information obtained for this metabolite is consistent with the identification of Peak V as 12-oxo-5,8,14-eicosatrienoic acid.

**Acknowledgements.** Supported in part by NIH grants EY 06513, HL34303, GM4 1206 and GM 3178.

**HPLC Separation of peak-V**

**PEAK V**

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CHARACTERISATION OF SYNTHETIC PEPTIDES
BY LASER DESORPTION MASS SPECTROMETRY

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The value of molecular weight measurement for characterising synthetic peptides has been amply demonstrated by the work of Chait's group at Rockefeller using $^{252}$Cf PDMS [1]. Out of a sample of 800 synthetic peptides from 15 different laboratories, almost half were found to be either inhomogeneous or incorrect! As Chait has observed, it is not surprising that many errors of synthesis go unnoticed when the technique most commonly used for assessing purity and identity, RP-HPLC, is the same technique as is used for purification.

Matrix Assisted Laser Desorption Mass Spectrometry (LD) provides an alternative to PDMS with certain advantages. LDMS is generally a more rapid and more sensitive technique, particularly for large peptides. The optimum sample loading for proteins and peptides is around 1 pmol, irrespective of mass, and a realistic time for sample preparation and analysis is 5 minutes. Mass measurement inaccuracy better than 0.1% is readily achieved for peptides and small proteins.

Matrix Assisted Laser Desorption Mass Spectrometry is also exceptionally useful in characterising modified and N-terminally blocked peptides which are not amenable to Edman sequencing.

The mass spectrometer consisted of a laser desorption ion source coupled to a Time-of-Flight mass analyser. All of the spectra reported here were obtained in positive ion mode. Negative (deprotonated) ions are produced by the ionisation process but, for these samples, the positive ion spectra were generally more intense.

Operating conditions and procedures were modelled on the work of Karas et al [2]. The sample concentration was adjusted to between $10^{-6}$ and $10^{-8}$ mol. Approximately 0.1 µl of sample solution was mixed with 0.5 µl of sinapinic acid matrix solution in the centre of a stainless steel target and the droplet allowed to dry at room temperature before introduction into the instrument. The matrix was dissolved in 70% acetonitrile, 30% water, 0.1% trifluoroacetic acid to give an approximate concentration of $5\times10^{-2}$ molar.


Example 1: The predicted sequence is RSFVCEVCTAROFARQENLKRHYRSHTNEK

The calculated average molecular weight for the protonated ion is 3568.0u. This species is present in the spectrum as a minor peak, but the major peak is at 3440u. The mass difference of -128u suggests a deletion product resulting from loss of either glutamine, lysine, or glutamic acid (-129u). This was later confirmed to be loss of glutamine by Edman sequencing.

Example 2: The predicted sequence is VERMVSPIESAEDC

The calculated average molecular weight for the protonated ion is 1565.8u. The major peak corresponds to the desired product, but there are also numerous other peaks present. The doublet at +90u and +105u may represent incomplete removal of a benzyl protecting group and a condensation product with thioanisole scavenger. It is interesting to observe the triplet in the vicinity of 1750u which represents the three permutations of peptide exhibiting both of these modifications: +90+90, +90+105, +105+105. There are also weak peaks at (MH+251) and (MH-78) which we are not able to assign.
Mass Spectrometric Signature of S-Prenylated Cysteine Peptides

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Protein prenylation is a recently discovered post-translational modification that has important ramifications for protein structure and function. For example, farnesylation of the RAS oncogene product is required for its membrane association and subsequent oncogenesis of the cell overexpressing the RAS protein. A large percentage of pancreatic and colon tumors express the RAS protein.  

Hitherto, identification and differentiation of S-prenyl cysteine derivatives has been a laborious process involving degradation of the thioether with Raney nickel, purification of the reaction mixtures, and identification of the hydrocarbon fraction by gas chromatography/mass spectrometry (GC-MS); a process requiring 150-200 µg of material.

SIGNATURE FRAGMENTS Examination of the FAB mass spectra (requiring only 2-5 µg of material) of thirteen synthetic analogs of the α-factor pheromone (H-Tyr-Ile-Ile-Lys-Gly-Val-Phe-Trp-Asp-Pro-Ala-Cys(S-farnesyl)-OCH₃) of Saccharomyces cerevisiae, revealed a consistent fragmentation which serves as a mass spectrometric signature of cystein prenylation. Fig. 1 displays two such spectra (FAB matrix = thioglycerol + 1% trifluoroacetic acid) whereby clusters A represent the protonated molecules, and clusters B and C the signature fragments. The mass difference A-B = n x 68 Dalton (n = number of isoprene units in the prenyl substituent) characterizes fragment B. The difference B-C = 34 Dalton characterizes fragment C. Only the observation of both fragments signals the presence of an S-prenylated cystein. The proposed mechanism for generating these fragments is presented in Fig. 2.
STRUCTURAL VARIATIONS

Variations in the nature of substituent Y (Fig. 2) did not effect the signature fragments substantially. Thus, Y = OCH₃, OC₂H₅, or NH₂ generated approximately the same abundances of fragments B, and C for identical peptide residues and prenyl chain lengths. By contrast, the intensities of the fragment clusters B and C (relative to A) were found to increase dramatically for smaller peptide residues, and to decrease somewhat for smaller prenyl chain lengths.

COLLISION INDUCED DISSOCIATION

Low energy CID (15 eV collisions with Ar at ca. 10⁻⁴ Torr) of the protonated molecules of prenylated cysteins having short peptide chains generated the signature fragments almost exclusively, whereas collision energies in excess of 50 eV produced numerous smaller fragments as well (Fig. 3).

References:

LIQUID SECONDARY ION MASS SPECTROMETRIC ANALYSIS OF
ABNORMAL PROTEINS: Hb Coventry

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Haemoglobin Coventry (Hb Coventry) is one of a group of abnormal or unstable human haemoglobins associated with mild haemolytic anaemia. Hb Coventry (B 141 leu deleted, B\textsuperscript{ Coventry}) has been reported on three previous occasions. In each case it was presented as a minor component which co-precipitated with another unstable haemoglobin during stability tests. Hb Coventry was first reported in association with Hb Sydney (B 67 Val\rightarrow Ala, B\textsuperscript{ Sydney})\textsuperscript{(1)}; later in association with α-Thal-1 (2 genes deleted)\textsuperscript{(2)} and more recently (3), with Hb Atlanta (B 75 Leu\rightarrow Pro, B\textsuperscript{ Atlanta}). Indeed in this latter case the propositus was found to have three different haemoglobin B chains, namely - B\textsuperscript{ Atlanta} (23%), B\textsuperscript{ Atlanta-Coventry} (11%) and B\textsuperscript{A} (normal, 66%). A re-investigation of the Hb Coventry by cDNA sequence analysis and by LSIMS analysis of the B\textsuperscript{ Coventry}-14 peptide arising from the tryptic digest of the aminoethylated B\textsuperscript{ Atlanta-Coventry} chain suggests that the modification arises from a post-translational change rather than from deletion of the leucine residue, B 141.

Experimental

Chain separations were performed in CM-Cellulose at pH 6.8 in 8M urea. Tryptic digestion, 2D- and reverse phase mapping were carried out using established procedures (3). Automated amino acid analysis was carried out with both ninhydrin detection and precolumn derivatisation with PITC. cDNA sequencing was performed using reverse transcriptase, polymerase chain reaction and direct sequence analysis. Automated amino acid analysis was carried out with both ninhydrin detection and precolumn derivatisation with PITC. cDNA sequencing was performed using reverse transcriptase, polymerase chain reaction and direct sequence analysis. Pure B\textsuperscript{A} and B\textsuperscript{ Coventry} peptides were hydrolysed with Chymotrypsin and then incubated with Carboxypeptidase A. Samples were analysed by LSIMS after 0,30,60,120 minutes incubation. 25keV LSIMS peptide mass spectra were obtained from a VG70-250S instrument fitted with a VG LSIMS ion source and associated caesium ion gun. Accurate mass measurement (RP 3000) of the protonated molecular ion was carried out using a software based multichannel analysis technique with CsI as external standard.

Results and Conclusion

Maps of the tryptic peptides were prepared from digests of isopropanol precipitated globin, total globin and isolated total B-globin. 2D peptide maps of total B- chains showed all the expected normal peptides. There were, however, three additional histidine-staining peptides present B\textsuperscript{A}9, B\textsuperscript{A}8-9 and B\textsuperscript{A}14. This analysis was repeated using the corresponding peptides from the same digests following HPLC separation (Figure 1). Repeated peptide amino acid analysis of B\textsuperscript{ Coventry} showed a composition identical to B\textsuperscript{A} except for the absence of the only leucine residue. There was no evidence of a substituted amino acid or proline or tryptophan. From this data it was concluded that the B-14 peptide from Hb Coventry was B 141 Leu deleted in agreement with previous studies.

Recent cDNA sequence analysis has shown that the mRNA and gene giving rise to the B-globin protein in Hb Coventry does code for the B 141 Leu residue and that this region of the gene is identical to that coding for normal B globin.

LSIMS of the B\textsuperscript{A}14 (1150 daltons) and B\textsuperscript{ Coventry}14 (1166 daltons, Figure 2) tryptic peptide either measured directly from 2D electrophoretograms by mounting the paper spot onto the LSIMS target or by analysing the corresponding HPLC fraction gave identical results. Accurate mass measurement of B\textsuperscript{ Coventry}14 and B\textsuperscript{A}14 using CsI and gramicidin S as external standards gave masses of 1165.5848 daltons and 1149.6501 daltons, respectively.

To investigate the primary sequence further, the B\textsuperscript{ Coventry} dodecapeptide was digested with Chymotrypsin to give an isolatable nonapeptide (829 daltons) in which the carboxy terminus was shown to be the post-translationally modified residue (designated Leu-X). Subsequent hydrolysis with Carboxypeptidase A for different times up to 2 hours gave an LSIMS map (Figure 3) showing the sequential loss of Leu-X (700
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

daltons), Ala (629 daltons) and Asn (515 daltons).

$\beta^{397\text{ery}}_{14}$ peptide: $^{133}\text{Val-Val-Ala-Gly-Val-Ala^*-Asn^*-Ala^*-Leu^*-Ala-His-Lys}^{144}$

(a = Carboxypeptidase A hydrolysis sites; c = Chymotryptic hydrolysis site)

These results indicate that Hb Coventry is not due to a deletion of a Leu residue at $\beta$ 141. Instead the residue at $\beta$ 141 was found to be 16 daltons higher than Leu. This suggests Hb Coventry arises from a post-translational change at $\beta$ 141. Work is continuing to characterise Leu-X.

References


Figure 1: HPLC reverse phase separation of the tryptic peptides from globin.

(a) - normal lysate. (b) - Hb Coventry.

Figure 2: Low resolution LSIMS spectrum for $\beta^{397\text{ery}}_{14}$.

Figure 3: LSIMS map of the peptide mixture from Carboxypeptidase A hydrolysis (2 hr) of the 829 dalton Chymotryptic peptide obtained from $\beta^{397\text{ery}}_{14}$.
Fatty acyl coenzyme A (acyl-CoA) thioesters are essential intermediates in all aspects of fatty acid metabolism: synthesis, degradation, and lipid esterification. Long chain acyl-CoA compounds have also been implicated in a number of pathological conditions where increased concentrations may lead to cellular injury.

Recently it was shown that arachidonoyl-CoA inhibits platelet activating factor (PAF, 1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine) synthesis in bovine neutrophil cell homogenates but free arachidonic acid had no effect on PAF synthesis (2). Also, trilacosin (an inhibitor of acyl-CoA synthetase) is reported to potentiate PAF production in rat polymorphonuclear leukocytes (3). Studies from our laboratory have shown a modulating effect on the synthesis of PAF in stimulated neutrophils due to exogenously added fatty acids. The fatty acid specificity of PAF synthesis inhibition suggested a role for long chain fatty acyl-CoA acyltransferase in competition with acetyl-CoA acetyltransferase in the utilization of 1-hexadecyl-CoA (1). Recently it was shown that arachidonyl-CoA inhibits 

Sonic-3-phosphocholine) synthesis in bovine neutrophil homogenates but free arachidonic acid had no effect on PAF synthesis. Also, trilacosin (an inhibitor of acyl-CoA synthetase) is reported to potentiate PAF production in rat polymorphonuclear leukocytes.

Methods

Standard long-chain acyl-CoA compounds were obtained from Sigma (St. Louis, MO). All standards were dissolved in methylene chloride:methanol (2:1) to give a final concentration of 1 ug/ul. Aliquots (1 ul) were applied dissolved in methylene chloride:methanol (2:1) to give a fatty acyl-CoA acyltransferase in competition with acetyl-CoA acetyltransferase in the utilization of 1-O-hexadecyl-sn-glycero-3-phosphocholine, the immediate precursor to PAF. For this reason, we investigated the negative ion FAB/MS behavior of long chain fatty acyl-CoAs and developed tandem FAB/MS/MS and CID protocols for the analysis of both specific acyl-CoAs and complex mixtures.

Coenzyme A Diagnostic Ions

A m/z 766 [CoA-H]+
B m/z 428 [3',5'-ADP-H]+
C m/z 408 [Acyl-CoA]+
D m/z 357 [phosphopantetheine-H]+
E m/z 339 [Acyl-2H2O]+

For identification of the specific acyl thioester, the second series of ions which retain the acyl group are more important.

Fatty Acyl Diagnostic Ions

I m/z 875 [acyl diphasophopantetheine-H]+
II m/z 857 [ion [H2O]+
III m/z 595 [acyl phosphopantetheine-H]+
IV m/z 577 [ion [H2O]+
V m/z 571 [C15H27CO]+, acyl thiolate anion

Negative ion FAB/MS produced more abundant ions which retained the acyl group than the reported positive ion spectra (4,5). This prompted further study of their CAD behavior to evaluate specific decompositions for the analysis of complex mixtures.

Tandem FAB/MS/MS and CID of Acyl-CoAs

The most abundant product ions from the collision induced decomposition of [M-H]+ ions were B and C, ions which contained only the diposphoadenosine structure. As these ions were not very abundant in FAB/MS and do not retain any acyl information they were not considered suitable for the analysis of complex mixtures or specific acyl-CoAs.

The product ion spectra of III and IV (m/z 595 and 577 for palmitoyl-CoA) contained very abundant ions at m/z 357 and 339 (ions D and E), respectively, corresponding to the loss of alkylketene. Although these ions also do not retain any acyl information, appropriate precursor and neutral loss protocols were developed to take advantage of this very favorable decomposition.

A mixture of seven long-chain fatty acyl-CoAs were analyzed by both single and tandem FAB. The single FAB/MS spectrum (Fig. 2) displayed quite a mixture of ions. However as the ion at m/z 339 (ion E) is common to all acyl-CoAs, the tandem FAB/MS/MS analysis of the precursors of m/z 339 yields a clear series of ions which are diagnostic of the molecular acyl species present (Fig. 3).

Likewise, the same decomposition can be used to analyze for a specific acyl-CoA. As this fragmentation involves loss of the alkylketene from the acyl group, a tandem mass spectrometric analysis for the neutral loss of a specific alkylketene will produce a spectrum diagnostic for that acyl-CoA.

References

Figure 1. Structure and negative ion FAB/MS of palmitoyl CoA (16:0-CoA).

Figure 2. Negative ion FAB/MS of 16:0-, 17:0-, 18:1-, 18:0-, 19:0-, 20:0-, and 20:4-CoA.

Figure 3. Tandem FAB/MS/MS of precursors of m/z 339 for the fatty acyl-CoA mixture shown in Figure 2.
Analysis of Acylcarnitines as their N-Demethylated Ester Derivatives by Gas Chromatography-Mass Spectrometry

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Carnitine is essential for the transport and metabolism of long-chain fatty acids. It is also involved in the detoxification of specific short- and medium-chain acyl groups that are poorly metabolized. In certain disease states, quantitative and qualitative analysis of urinary acylcarnitines enable the recognition of metabolic defects that may not be easily detected by organic acid analysis [1]. The analysis of acylcarnitines has been difficult because of the presence of a quaternary ammonium group and the resulting involatility. Presently available procedures, mostly based on HPLC, FAB-MS and TSP-HPLC-MS, generally have limitations in specificity and sensitivity, or require specialized equipment [2]. Recently, a procedure was reported to overcome these limitations [3] which involved the conversion of acylcarnitines into corresponding volatile acyloxybutyrolactones followed by GC-MS analysis.

We report here a new, simple procedure in which the acylcarnitines are analyzed as the N-demethylcarnitine esters (R’= Et, Pr) by a two-step derivatization followed by GC or GC-CI(methane)-MS.

![Chemical Structure](attachment:image.png)

**DERIVATIZATION**

**Step 1.** Direct esterification by extractive O-alkylation with Et or Pr chloroformate in aq. EtOH (or PrOH) in the presence of pyridine.

**Step 2.** Ion-pair extraction using KI-chloroform to remove the acylcarnitine ester iodides into organic phase.

An aliquot of the above derivative is directly injected onto the GC-column to effect N-demethylation. Consequently, the mass spectra of the thermolytic products are registered as corresponding acyl N-demethylcarnitine Et (or Pr) esters (ADMCEE or ADMCPE). For urine samples, the derivatization is preceded by purification through a column of AG 1X8 anion exchanger followed by enrichment using a C-18 cartridge.

**INSTRUMENT** Spectra were acquired on a JEOL AX 505H double focusing mass spectrometer coupled with a HP 5890J gas chromatograph. GC-MS conditions: DB-1 column (30 m x 0.25 mm x 0.25*m film coating) programmed at 5 or 10 C/min between 100-300 C, or 150-300 C. He gas flow 1 ml/min. Reagent gas methane. Scan rate 50-500 Daltons in 1 s/scan.

**RESULTS** The dequaternized derivatives exhibited well-separated peaks in the chromatogram. Distinctive mass spectra were recorded that permitted the chain features of individual molecular species, including those of isomeric compounds to be distinguished. Figures presented below are gas chromatogram and selected CI mass spectra of derivatized acylcarnitines which were obtained from the urine of a patient suffering from medium-chain acyl-CoA dehydrogenase (MCAD) deficiency.

**CONCLUSION** The present method, owing to the extreme simplicity and unambiguous characterization it can achieve, may constitute a potentially useful tool in the study of fat metabolism. The advantages include:

1. Simple operation, applicable to carnitine esters derived from mono- and dibasic acids.
2. Good GC separation and distinctive mass spectra allow qualitative and quantitative analysis of mixtures.
3. Nanogram detection, suitable for analysis at physiological levels.
4. High overall efficiency of sample processing: The analysis of a urine sample can be completed within 1 h - 15 min for urine pretreatment, 15 min for derivatization, and 15-30 min for GC-MS analysis.

REFERENCES

**Selected Spectra of Acylcarnitine Derivatives Found in the MCAD Deficiency Urine**

<table>
<thead>
<tr>
<th>Peak [M+H] m/z</th>
<th>Structure</th>
<th>Peak area</th>
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<tbody>
<tr>
<td>274</td>
<td>5:0 DMCPE</td>
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<tr>
<td>288</td>
<td>6:0 DMCPE</td>
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<td>Valproyl DMCPE*</td>
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* Valproylcarnitine was used as an internal standard (10 μg/0.5 ml urine)
DETECTION OF 2-HYDROXY-3-METHYLVALERIC ACID ENANTIOMERS
BY CAPILLARY GC/MS

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Montreal, Quebec, Canada H3A 1A3

Introduction. Elevated levels of 2-hydroxy-3-methylvaleric acid (HMVA) in body fluids are symptomatic of maple syrup urine disease (MSUD), a disorder resulting from any of several inherited defects in the multi-enzyme complex, branched chain alpha-keto acid dehydrogenase. Earlier GC/MS studies of branched-chain keto and hydroxy acids were limited by poor chromatographic resolution through the use of packed columns. We now present the analysis of HMVA as its silylated derivative on a DB-1 capillary column coupled directly to a quadrupole mass spectrometer; the improved chromatography allows the resolution of two diastereomeric pairs of HMVA (R,R; S,S and R,S; S,R) in urine. Reaction with a chiral reagent resolves the four enantiomers of racemic HMVA on a non-chiral DB-1 column.

Sample Preparation. Racemic HMVA and D$_2$-HMVA were prepared by the reduction of KMVA with NaBH$_4$ or NaBD$_4$, respectively, in a basic (pH 12) aqueous solution. Extraction of organic acids from both MSUD urine and standards was as follows: a 2 ml aliquot was made basic (NaOH; pH 12), saturated with NaCl and extracted twice with diethyl ether (ether layers discarded); the sample was acidified (HCl; pH 2), extracted three times with ether and dried over Na$_2$SO$_4$. The chiral derivatization was carried out by reacting HMVA with an excess of (S)-(++)-2-methylbutyric anhydride (MBA) in pyridine at 60°C for 3 h and extracting as described above. TBDMS derivatives of HMVA and D$_2$-HMVA and the TMS derivative of HMVA-MBA were prepared by standard methods.

Instrumental. GC/MS analyses were performed using an HP 5890A gas chromatograph directly coupled to an HP 5988A quadrupole mass spectrometer. Samples were introduced by splitless injection onto a fused silica DB-1 capillary column heated from 100 to 200°C at 5°C/min. El mass spectra were acquired under standard conditions while NH$_3$/CI spectra were obtained at a source pressure of 0.45 Torr and an ionizing energy of 150 eV.

Results and Discussion. Fig. 1 depicts the El mass chromatograms (m/z 303) for the TBDMS derivatives of (a) the MSUD urine extract and (b) authentic HMVA. The corresponding El mass spectra for the four GC eluates are shown in Fig. 2. The excellent correlation in retention times and mass spectra confirm that the MSUD urine extract contains the two diastereomeric forms of racemic HMVA. Some of the major ions in the El mass spectrum of HMVA-(TBDMS)$_2$ ([M]+, m/z 360) are: [M-56]+ (m/z 304, loss of C$_4$H$_9$ from acid backbone), [M-57]+ (m/z 303, loss of t-butyl from TBDMS group), [M-85]+ (m/z 275, loss of CO from m/z 303) and [M-159]* (m/z 201, loss of CO$_2$TBDMS). These fragmentations were substantiated by the analysis of D$_2$-HMVA-(TBDMS)$_2$ ([M]+, m/z 362), which gave prominent ions at m/z 305 ([M-57]+), m/z 277 ([M-85]+) and m/z 203 ([M-159]*). Molecular weight confirmation was obtained by the NH$_3$/CI analysis of the urine extract, giving prominent [M + NH$_4$]+ (m/z 378) and [M + H]+ (m/z 361) ions. The El mass chromatogram (m/z 171) for the TMS derivative of the HMVA-MBA complex ([M]+, m/z 288) is depicted in Fig. 3 and shows partial resolution of the four HMVA enantiomers. The corresponding mass spectra reveal the following major ions: [M-14]+ (m/z 274, loss of CH$_2$), [M-15]+ (m/z 273, loss of methyl from TMS group), [M-57]+ (m/z 231, loss of C$_4$H$_9$ from acid backbone), [M-89]+ (m/z 199, loss of -OTMS) and [M-117]+ (m/z 171, loss of CO$_2$TMS). This study demonstrates the potential for performing conformational analyses on optically active metabolic products using simple and proven sample preparation methods and readily available GC/MS instrumentation.

Acknowledgements. This work was supported in part by the Medical Research Council of Canada.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Figure 1. (a) EI mass chromatogram (m/z 303) for the TBDMS derivative of MSUD urine extract. (b) EI mass chromatogram (m/z 343) for the TBDMS derivative of HAVL.

Figure 2. EI mass spectra of GC eluates depicted in Figure 1.

Figure 3. (a) EI mass chromatogram (m/z 171) for trimethylsilyl (t-Boc-1,3,5-trimethylbenzyl)3-bromo-3-methylpentanoate. (b) - (e) EI mass spectra of components 1 - 4, respectively.
URINARY ORGANIC ACID PROFILE STUDIES IN A PATIENT WITH CYTOCHROME C OXIDASE DEFICIENCY

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Introduction

As causes of chronic lactic acidosis, abnormalities of the pyruvate dehydrogenase complex, abnormalities of gluconeogenesis, the respiratory chain disorders and other disorders including organic acidurias are known.

A number of patients with disorders in the respiratory chain affecting mitochondrial respiration and manifested clinically as a mitochondrial myopathy have been reported.

We have studied the urinary organic acid profiles in a female infant with cytochrome C oxidase (CCO) deficiency by capillary gas chromatography-mass spectrometry. The urinary organic acid profiles of a patient with glucose-6-phosphatase deficiency and one with fructose-1,6-diphosphatase deficiency during hypoglycemic attacks were also examined. These organic acid profiles were clearly different from those of patients with the respiratory chain disorders.

The urinary acid profiles of a patient with pyruvate carboxylase deficiency, during a ketotic attack and during remission, were also investigated.

Methods

To urine equivalent to 0.5 mg creatinine, 20 μg each of 3-hydroxymyristic acid and heptadecanoic acid was added, after acidification to pH 1.0 with 6N HCl. The urinary acids were extracted with ethylether three times and converted to their trimethylsilyl derivatives.

A JEOL JMS DX-300 gas chromatography-mass spectrometer supported by a JMA 3500 data acquisition system was used for GC/MS analysis. A MPS-50 fused-silica-capillary column (25m x 0.32 mm i.d., 1.0 μm film thickness, Quadrex Corporation, Connecticut, USA) was used for the chromatographic separation.

Results and Discussion

The urinary organic acid profiles in a case with cytochrome C oxidase (CCO) deficiency (case 1) were always characteristic of those found in the disorders in the electron transport system: markedly increased excretion of lactate and 2-hydroxybutyrate, with an increased serum lactate/pyruvate ratio and frequently with an increased 3-hydroxybutyrate/acetoacetate ratio.

Citrate, malate, fumarate and 2-oxoglutarate were all increased. 2-Hydroxyglutarate/2-oxoglutarate ratio was also increased. In addition p-hydroxyphenylacetate, the reduced form of a tyrosine catabolic intermediate (p-hydroxyphenylpyruvate), was also significantly increased. Fumarate/succinate ratio was also elevated.

The disorders of gluconeogenesis (pyruvate carboxylase deficiency, glucose-6-phosphatase deficiency, fructose-1, 6-diphosphatase deficiency, during clinical attacks) showed quite different metabolic profiles from those of CCO deficiency. During a ketogenic attack in pyruvate carboxylase deficiency, lactate and ketone bodies were dramatically increased but citrate, malate, 2-oxoglutarate, 2-hydroxyglutarate, succinate and fumarate were markedly decreased. The amino acids, asparagine and glutamine which are metabolically very close to the TCA cycle, were also greatly decreased. The 3-hydroxybutyrate/acetoacetate ratio was dramatically increased. After citrate
supplementation for the purpose of providing TCA-cycle-substrates, these TCA-cycle-related amino acids and TCA-cycle intermediates were increased to the control level.

During the hypoglycemic attack in glucose-6-phosphatase deficiency and fructose-1,6-diphosphatase deficiency, lactate, ketone bodies and TCA-cycle intermediates were significantly increased. The increase in 2-oxoglutarate was most impressive. Fumarate was also greatly increased; however, the 2-oxoglutarate/fumarate ratio was also increased in these two disorders of gluconeogenesis. Dicarboxylic acids (adipate and suberate) were not increased in any of the three disorders of gluconeogenesis.

Conclusion

The present investigation showed that the urinary organic acid profiling is useful for the differential diagnosis of chronic lactic acidosis which is critically important for the correct treatment of patients with lactic acidosis.

Organic acid patterns in urine of patients with congenital lactic acidosis
- an aid to the differential diagnosis -

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\( \mu g/mg \) creatinine
RAPID DIAGNOSIS OF DISORDERS IN AMINO ACID METABOLISM; QUANTITATIVE ANALYSIS OF SPECIFIC AMINO ACIDS IN SMALL BLOOD SAMPLES BY FAB MS/MS.

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Tandem mass spectrometry (MS/MS) coupled with fast atom bombardment ionization (FAB) is well suited for the rapid analysis of complex biological samples. Numerous compounds of various structural classes can be analyzed with excellent selectivity and specificity without the need for time-consuming chromatographic separations. This laboratory routinely uses FAB MS/MS techniques for the detection of abnormal urine and plasma metabolic profiles in patients with inborn errors of metabolism. We report here a new application of this technique to screen for disorders of amino acid metabolism such as phenylketonuria (PKU) and maple syrup urine disease (MSUD). Quantitative analysis using isotope dilution has been developed for several amino acids in plasma and small blood spots on filter paper.

Preparation of plasma and blood spot samples was as follows. Two hundred μL of plasma was deproteinized with ethanol. A 6 mm diameter circle containing approximately 12 μL of blood was punched from a PKU card (Guthrie card) and mixed with 1 mL of methanol. Internal standard was added to each sample and the contents were vortexed (PKU card allowed to stand for 30 minutes), dried, and butylated with 3 M HCl-butanol at 65°C for 20 minutes in a capped vial. After this, the mixture was dried and reconstituted in 50 μL of methanolicoliglycerol. Five μL of final sample solution were placed on the FAB probe tip for analysis. Two triple quadrupoles were used, a VG QUATTRO with Lab-base data system and a VG TRIO-3 with DEC 11-250 data system. The TRIO-3 used a sledge-field FAB gun to generate 8 Kev xenon atoms and the QUATTRO used a cesium ion gun operated at 10 Kev. Argon was used as the collision gas and the collision energy optimized. All MS/MS spectra (daughter and neutral loss scans) were recorded in 'multi-channel analysis' mode (60 consecutive scans, 1 sec/scans) at unit mass resolution and accumulated in a single raw spectrum.

The butyl ester derivative of amino acids provided satisfactory results (high sensitivity, few Interfering peaks) for their detection in blood or plasma as compared with MS/MS for non-derivatized amino acids or their methyl esters. The CID spectra of the MH+ ion of many amino acids are characterized primarily by a common neutral loss of the butyl ester group (102 daltons). For LYS, ARG, GLN, and ASN, the loss of ammonia (m/z 17) is more prominent. For these amino acids loss of 102 (butyl ester) occurs in addition to loss of ammonia. Therefore, these are detected in a neutral loss of 102 scan as an [MH+ - 17] ions. The scan function employed to generate the amino acid profiles was the constant neutral loss of 102 daltons. This profiling technique was applied to analysis of 200 μL aliquot of pooled plasma from normal patients Figure 1a. The lack of chemical Interference is notable and suggests feasibility for quantitation of several amino acids by isotope dilution mass spectrometry. Samples were also analyzed from blood spots placed on filter paper. Amino acid profile obtained from a 6mm spot of blood cut from a normal patient is presented in Figure 1b. A strikingly similar pattern with less chemical interference is observed relative to the plasma profile (Figure 1a).

Simple isotope dilution assays for leucine, phenylalanine, and tyrosine were developed in using D3,Leu, D3-Phe, and D3-Tyr as internal standards. A calibration curve was derived from analysis of standard solutions containing varying amounts of labeled amino acid in the presence of internal standards. The results showed excellent linearity for each amino acid with correlation coefficients of 0.99 or greater. The detection limits for LEU, PHE, TYR were less than 25, 5, and 45 nmol/mL, respectively. LEU, PHE, and TYR were quantified in 200 μL plasma samples or 6 mm spot from a PKU card from normal patients. The ratios of peak areas were obtained after using a neutral loss scan over a limited mass range. The results from the analysis of 10 normals by FAB MS/MS were compared with those obtained from a Beckman model 7300 amino acid analyzer and the data were in close agreement with each other (10%).

The quantitative determination of phenylalanine is important in the detection of phenylketonuria (PKU) a metabolic disorder characterized by a deficiency or absence of phenylalanine hydroxylase, an enzyme which converts phenylala-nine to tyrosine. The result of this disorder is elevated phenylalanine and depressed tyrosine concentrations in plasma and urine. The State of North Carolina currently screens all infants for PKU. Their method only quantifies the concentration of phenylalanine by a fluorometric assay. However, elevated phenylalanine may also present in certain liver metabolism disorders in addition to other amino acids. A more specific test for PKU would be a method which quantifies the phenylalanine to tyrosine ratio. FAB MS/MS has this capability. Several PKU cards were sent to us from the State of North Carolina screening program and categorized as normals, known PKU, and false positive (by their method). The samples were quantified by FAB MS/MS and the mole ratios of PHE/TYR were determined. It was found that the PHE/TYR ratio for normals was quite distinct for known PKU patients as compared to normals or false positives.

An amino acid profile of a known PKU patient obtained from 6mm blood spot from a PKU card is presented in Figure 1c. The quantitation of leucine/isoleucine (LEU/ILE) resulted in the detection of Maple Syrup Urine Disease (MSUD) which is characterized by highly elevated leucine concentration in plasma. An amino acid profile of one patient with MSUD is presented in Figure 1d. The concentration of LEU/ILE is over 2000 nmol/mL (normals < 250 nmol/mL).

The results presented here suggest the general applicability of FAB MS/MS for diagnosis of metabolic disease. This technique is rapid and is amenable to large scale automated analysis and screening. If combined with acylcarnitine profiles now currently performed in this laboratory, the number of metabolic disorders which could be detected in one test is greater than 15. MS/MS is so versatile that new classes of compounds may be potentially screened. The authors would like to acknowledge the following: Grants from NICHD Division of the NIH (HD-24908) and the State of North Carolina, Division of Maternal and Child Health. We also thank Fisons-VG Biotech for the temporary loan of a TRIO-3 tandem quadrupole mass spectrometer.
Figure 1A: Neutral loss scan of 102 mass units by FAB MS/MS from 200 µL of pooled plasma of normal patient. An internal standard, D₃-LEU (80 nmol) was added to 200µL of plasma. B: Neutral loss scan of 102 mass units by FAB MS/MS from a 6 mm blood spot punched from a PKU card from a normal patient. The internal standards, D₃-LEU 1 nmol, D₅-PHE 1 nmol, and D₃-TYR 1 nmol, was added to the spot which is equivalent to 6 µL of plasma. C: Neutral loss scan of 102 mass units by FAB MS/MS from a 6 mm blood spot punched from a PKU card from a patient with phenylketonuria (PKU). The internal standards, D₃-LEU (1 nmol), D₅-PHE (1nmol), and D₃-TYR (1 nmol) was added to the spot which is equivalent to 6 µL of plasma. D: Neutral loss scan of 102 mass units by FAB MS/MS from 200 µL of pooled plasma of an MSUD patient. An internal standard, D₃-LEU (80 nmol) was added to 200µL of plasma.

Lysoplasmenylethanolamine (1-0-alkenyl-sn-glycero-3-phosphoethanolamine, also denoted as lyso-VEPE for lyso vinyl ether phosphoethanolamine) and its diacetyl analogs (diacetyl-VEPE) are being utilized in our laboratories for studies related to Platelet-Activating Factor (PAF). When fast atom bombardment mass spectrometry was used during our development of new methods for high purity preparation of these phosphoglycerides (Hanahan, D.J., Nouchi, T., Weintraub, S.T. and Olson, M.S. J. Lipid Res. 31, 2113-2117, 1990), we observed several interesting features about the spectra. Not only did acetylation strongly influence the nature of the spectrum, but a chemical reaction between the thioglycerol matrix and the analyte was also found to occur.

FAB mass spectra of lyso-VEPE, using a matrix of either glycerol or thioglycerol, are shown below.

![FAB mass spectra of lyso-VEPE](image)

In both cases, distinct \([M + H]^+\) ions can be seen for each molecular species. Note that the fragmentation patterns in both spectra are quite similar, except that the ions at m/z 331, m/z 357 and m/z 359 are only present when thioglycerol is the matrix. From the mass separation and overall appearance of this ion cluster we suspected that the alkenyl side chains of the VEPE homologs were included in these fragments. We hypothesized that in the slightly acidic environment of thioglycerol, fatty aldehyde could be liberated which could then react with the matrix to form the compound shown on the next page. Alternatively, direct attack of thioglycerol on the vinyl ether might occur. In order to confirm the composition of the reaction product, accurate mass analysis was undertaken. The results for the ion at m/z 357 are: measured mass, 357.2836; formula, \(C_{21}H_{41}O_2S\); difference from calculated value, 0.9 mmu (2.5 ppm).
Further insight into the nature of these ions was gained by exposing lyso-VEPE to strong acid and then analyzing the liberated fatty aldehyde by FAB/MS. No ions above background were observed when a glycerol matrix was utilized, but in thioglycerol, the majority of the ion current was found in the m/z 357 base peak. While the composition of the fatty aldehyde released from the lyso-VEPE has not been ascertained through dimethylacetal formation followed by GC/MS analysis, the ion at m/z 357 is consistent with the protonated reaction product of thioglycerol with 18:1 aldehyde.

During these studies we have also been investigating the effects of acetylation on the ability of lyso-VEPE to inhibit PAF-induced platelet aggregation and secretion. From FAB/MS of the diacetyl derivatives we observed that there were much greater differences in the fragmentation patterns as compared to that of lyso-VEPE than just the addition of 42 or 84 mass units to appropriate ions. In the spectrum of our first batch of diacetyl-VEPE, [M + H]+ ions for each molecular species were clearly detectable in addition to lower intensity [M + K]+ ions. However, in subsequent preparations, [M + H]+ ions were essentially not detected, and [M + K]+ increased in relative intensity. In addition, the ion at m/z 222, representing potassium cationized acetylphosphoethanolamine, was the base peak. It was interesting to note that the ions at m/z 331, m/z 357 and m/z 359 were detected in the diacetyl analogs, albeit at lower intensity in the more highly potassium cationized samples.

We presume that the K+ became associated with the lyso-VEPE as a consequence of the alkaline methanolysis with KOH (even though ions representing either [M + K]+ or thioglycerol-potassium adducts were not seen in the FAB mass spectrum of lyso-VEPE). Not only have we been unable to determine why our initial sample differed somewhat from later preparations, but we have also not been able to dislodge the potassium without destroying the vinyl ether moiety.

From an examination of lyso-phosphatidylyethanolamine (lyso-PE) and its diacetyl derivative, we found that the enhanced attachment of potassium was not limited to vinyl ethers alone. Similarities with the between the FAB spectra of diacetyl-PE and diacetyl-VEPE were clearly seen, in particular, an increase in intensity for the ion at m/z 222, and a decrease for the ion at m/z 117.

We conclude that vinyl ether phosphoethanolamines can react with thioglycerol to form a covalent compound between the matrix and the alkenyl side chain. A comparable reaction does not occur when acyl or ether side chains are present. In addition, acetylation of the ethanolamine moiety significantly affects ion binding to the phosphate, resulting in strong complexation with adventitious potassium ions which remain associated after alkaline methanolysis.

ACKNOWLEDGEMENTS - These studies were supported in part by grants from the National Institutes of Health (DK-33538) and the Robert A. Welch Foundation (AQ-0887). We are grateful to Dr. Douglas Gage for the accurate mass analyses performed at the Michigan State University NIH Mass Spectrometry Facility (NIH grant number RR-00480). Thanks are also due to Terri Nouchi for expert technical assistance.
CAD FRAGMENTATION OF TAUROCONJUGATED BILE ACIDS AT LOW ENERGY

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FAB MS/MS negative ion spectra at low collision energy of bile salts mixtures from biological samples are useful in the diagnosis of several metabolic disorders\(^1\). A better understanding of the fragmentation pathways will allow to get more structural information from these spectra. We have thus studied the fragmentation of tauroconjugated bile acids anions with a Finnigan MAT TSQ70 triple quadrupole instrument. Xenon was used as a collision gas at .8 mTorr uncorrected gauge pressure reading.

Tomer et al.\(^2\) have described remote charge site fragmentations occurring at high energy CAD such as depicted by A to F in figure 1. We have observed that the FAB spectra of pure bile salts display the same remote charge site fragments (figure 2).

CAD spectra at 15 eV collision offset voltage also display remote charge fragments of the rings, but only when unsaturations and heteroatoms are present. Thus, taurotriketocholanic acid (figures 3 and 4) and tauroconjugated \(\Delta 4\) unsaturated bile salts display strong remote charge site fragments.

Saturated bile salts with hydroxyl groups display fragments corresponding to parts of the side chain and others corresponding to neutrals lost from the side chain, indicated by arrows in figure 1. These appear to be remote charge site fragments too, but with the charge now located on an hydroxyl group rather than on the taurine moiety. Changing the number or the position of the hydroxyl groups changes the relative intensities of these fragments. Moreover, additional hydroxyl groups induces losses of water molecules from these fragments, as illustrated in the figures 5 and 6.

In conclusion, fragments from the side chain are always present, and this allows for example selective detection of tauroconjugates by scanning the parents of 124 amu. Remote charge site fragments of the rings are observed at low collision energy when unsaturations, either as ring double bonds or keto groups, are present. Otherwise, the main observed fragments result from neutrals lost from the side chain. They differ in relative intensities for positional isomers, but are identical for geometric ones. For example, taurine conjugates of chenodeoxycholic (3,7\(\alpha\) diOH) and ursodeoxycholic (3,7\(\beta\) diOH) display the same spectrum, while deoxycholic (3,12\(\alpha\) diOH) display a very different one.
TAUROLITHOCHOLIC ACID
MW 483 uma

FIGURE 1: Fragmentation scheme of taurolithocholic acid. Taurochenodeoxycholic has an additional hydroxyl group at position 7 alpha.

TAUROTRIKETOCHOLANIC ACID
MW 509 uma

FIGURE 3: see spectrum fig. 4

TAUROLITHOCHOLIC ACID DAUGHTER OF 482 amu

FIGURE 5: See arrows in fig. 1. 416 = 482–66 amu, H₂SO₄; 396 = 416–18; 388 = 482–94; 374 = 482–108; 357 = 482–125

TAUROCHENODEOXYCHOLIC ACID DAUGHTER OF 498 amu

FIGURE 6: See fig. 1. Same neutral losses as in fig. 5, but with additional water losses.

DEVELOPMENT OF DYNAMIC FAB-MS TECHNIQUES FOR DETECTION OF DISORDERS OF STEROL AND BILE ACID METABOLISM

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INTRODUCTION
The usefulness of negative ion FAB-MS as an efficient screening tool for metabolic disorders affecting the conversion of cholesterol to bile acids is well established (1,2). Because this technique relies upon the appearance of only a few pathognomonic, pseudomolecular ions from the analysis of a urine extract for detection of a disorder, it is susceptible to interference from other extract components. Confirmation is usually accomplished by use of GC, GC-MS or thermospray LC-MS. This is a report of the use of CF-dynamic FAB-MS for either a rapid screening of patient urines by direct injection or for confirmation of findings by uHPLC-MS. Confirmation when a ketone or lactone containing compound is suspected is achieved by addition of NaBD₄ to the urine before extraction. This reduction is useful for elimination or confirmation of several isobaric, interfering or pathognomonic ions.

EXPERIMENTAL
Urine samples are extracted by a modification of the procedure of Lawson et al(1). A Brownlee Labs., Micro Gradient solvent delivery system is used for both direct injection CF-FAB/MS and uHPLC-FAB/MS with a VG 70-250 SE mass spectrometer. Chromatography is performed on a 1mm X 10cm uHPLC column packed with 3μm C-18 particles (Keystone Scientific). A gradient from 55% to 75% methanol in aqueous 0.1 M ammonium acetate in 40 min at 60 ul/min is used for these separations. The column effluent is split 1:20 with 3 ul/min going to the FAB probe. No sample splitting is used for direct injection analysis. Bile acid standards were obtained from Sigma Chemical Co.

RESULTS
Fig.1 demonstrates the use of direct injection CF-FAB/MS for screening of patient samples. One ul of urine extract (equivalent to 2ul of urine) was injected from 50% aqueous methanol containing 2% glycerol at a 6ul/min flow rate. Spectra from two of these injections are shown. This technique allows samples to be injected at 3 min intervals. Fig.2 is a standard curve for taurocholic (TCA) and glycocholic (GCA) acids from 0.5 to 15 ng injected with tauroursodeoxycholic acid (5ng) as an internal standard. A 5% coefficient of variation for 7 repeat injections of the sample containing 5ng was obtained.

Fig.3 shows the separation of a mixture of 13 standard bile salts obtained by uHPLC-CF/FAB. Also, shown are [M-H]⁻ plots for each component. Linear standard curves are obtained from 5 to 250ng injected using ursodeoxycholic acid as an internal standard.

Fig.4 shows the results of uHPLC-CF/FAB analysis of a urine extract from a patient with neonatal adrenoleukodystrophy under the conditions described above. Extracted ion plots for several pathognomonic bile salts are shown. The identification of the presumed isomers resulting in the multiple peaks seen in individual ion plots is currently being investigated.

REFERENCES

ACKNOWLEDGEMENT
This work was supported by a grant from the United Leukodystrophy Foundation.
DIRECT INJECTION STANDARD CURVES FOR TAUNO- & GLYCOCHOLATE

Fig. 1

STANDARD BILE SALTS

Fig. 3

FIG. 2

FIG. 4
Synthesis of $[\text{6,6,19-}^3\text{H}]\text{vitamin D}_3$ and quantitation of plasma vitamin D$_3$
by capillary gas chromatography-mass spectrometry.

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Childrens Hospital Research Foundation, Eland and Bethesda Avenues, Cincinnati, OH, U.S.A.

Introduction: Vitamin D and its metabolites play an important role in calcium homeostasis and are widely studied using assay techniques based on competitive binding or high performance liquid chromatography (HPLC). Reference methods based on stable isotope dilution with GC-MS analysis have been developed for quantifying several vitamin D metabolites in blood plasma, however no such method exists for vitamin D$_3$ itself. Most literature methods for labelling vitamin D and its metabolites rely on treatment of steroidal precursors with deuterated reagents such as CD$_3$/Mg. A simpler approach, in which vitamin D itself or any metabolite can theoretically be used as the starting material, was outlined by Yamada et al. (Tetrahedron Lett. 22:3085, 1981). We used a modified version of this method to synthesize $[\text{6,6,19-}^3\text{H}]\text{vitamin D}_3$. The product was characterized by several chromatographic and spectrometric techniques and was subsequently employed as the internal standard in the development of a GC-MS assay for vitamin D$_3$ in human plasma.

Methods:

Synthesis of $[\text{6,6,19-}^3\text{H}]\text{vitamin D}_3$: Vitamin D$_3$ (100mg) was refluxed with an excess of liquid SO$_2$ for 1h using a dry-ice condenser. Excess reagent was removed by aspiration and the SO$_2$ adduct (a mixture of C$_2$ epimers) was purified by chromatography on silica gel (28-200 mesh, 5g) using ethyl acetate as eluent. Hydrogen-deuterium exchange was performed at the activated C$_2$ and C$_3$ positions using 200µl deuterium oxide (minimum isotopic purity 99.996 atom % D, Aldrich Chemical Company, Milwaukee) in dimethylformamide (800µl) containing potassium t-butoxide (50mg). After stirring at room temperature for 1h the reaction was quenched by adding ice water and extracted with ethyl acetate. The extract was washed with brine, dried over Na$_2$SO$_4$ and evaporated to dryness. The deuterated adduct was purified on silica gel using ethyl acetate/methanol (99/1 v/v) as eluent. The adduct was then heated for 1h at 80°C under argon in a solution of sodium bicarbonate (5mg) in dimethylformamide (500µl) to drive off SO$_2$. After cooling, the mixture was diluted with ethyl acetate, washed, dried and evaporated. The resulting 5E-vitamin D$_3$ was purified on a small (2g) silica gel column eluted with ethyl acetate/hexane (1/1 v/v). The 5E-isomer was dissolved in ethanol/water (95/5 v/v) containing 2mg Eosin Y. This mixture was saturated with argon during a 2h irradiation with a 20W halogen lamp. The solvent was then evaporated and the product was again purified on silica gel. The final product was further purified by HPLC using a Hypersil 5µ C18 column (25 x 0.46 cm) eluted with methanol at a flow rate of 1ml/min with UV absorbance detection (264nm). The collected fraction corresponded to a partially resolved doublet on the chromatogram indicating the presence of a mixture of 5Z- and 5E-vitamin D$_3$ in a ratio of 6:4. The overall yield was relatively low (1.5mg).

Extraction of Vitamin D$_3$ from Blood Plasma:

1. Add $[\text{2H}]\text{Vitamin D}_3$ (40ng in 100µl methanol) to 1ml plasma
2. Add 2ml water and 1.5ml 2-propanol
3. Sonicate and equilibrate at room temperature
4. Prime a 8ml C18 Bond-Elut cartridge
5. Load sample under vacuum
6. Wash with 10ml water and 10ml methanol/water (85/15 v/v)
7. Elute with 4ml methanol and add 1ml water to the eluate
8. Wash the Bond-Elut cartridge
9. Load extract
10. Wash with 2ml methanol/water (85/15 v/v)
11. Elute with 6ml hexane/chloroform (95/5 v/v)

Extracts were further purified (to remove cholesterol) by two HPLC systems:

1) 25 x 0.46 cm Hypersil ODS column, eluted at 1ml/min with methanol/water (99/1 v/v) with UV absorbance detection (284nm)
2) 25 x 0.46 cm Whatman Partisil column, eluted at 1ml/min with hexane/2-propanol (98/2 v/v) with UV absorbance detection (284nm)

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GC-MS analysis:

- Derivatization: Plasma extracts and standards were converted to trimethylsilyl ether derivatives by heating with 50 μl 1% TMCS in BSTFA at 65°C for 15 min.
- Instrument: Finnigan 4535 quadrupole GC/MS system with Incos data system.
- Chromatography: A fused silica capillary column (30m × 0.25mm i.d., DB1 coated, J&W, Folsom, CA) was programmed from 250-320°C at 15°C/min and held at 320°C for 5 min.
- Mass spectrometry: The mass spectrometer was operated in electron ionization mode. Full scan spectra were recorded over the mass range m/z 50-800 every 2 s during the run. For quantitation the molecular ions of the trimethylsilyl ethers of vitamin D₃ and deuterated vitamin D₃ (m/z 456 and 459) were selectively monitored with a cycle time of 0.25 s.

Results:

<table>
<thead>
<tr>
<th>HPLC System 1 (reverse phase)</th>
<th>HPLC System 2 (normal phase)</th>
<th>Capillary GC (as TMS-ethers)</th>
<th>UV spectrum</th>
<th>λ_{max} (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>retention vol. (ml)</td>
<td>retention vol. (ml)</td>
<td>retention time (minutes)</td>
<td>M⁺ = m/z 459, isotope ratio [^{1}H];[^{2}H] (m/z 456:m/z 459) = 0.031</td>
<td></td>
</tr>
<tr>
<td>Authentic vitamin D₃</td>
<td>8.9</td>
<td>9.5</td>
<td>7.5</td>
<td>256.0</td>
</tr>
<tr>
<td>[6,6,19-'H₃]vitamin D₃</td>
<td>9.1</td>
<td>9.4</td>
<td>7.5</td>
<td>264.5</td>
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</tbody>
</table>

- Electron ionization mass spectrum of [6,6,19-'H₃]vitamin D₃ trimethylsilyl ether

Measurement of vitamin D₃ in human plasma:

<table>
<thead>
<tr>
<th>Calibration curve</th>
<th>Selected ion monitoring chromatograms from human plasma extract</th>
</tr>
</thead>
</table>

Summary and Discussion:

A sample of [6,6,19-'H₃]Vitamin D₃ was prepared by deuterium exchange into the s-cis diene sulphur dioxide adduct of vitamin D₃. The product was of high isotopic purity ([^{1}H₃]:[^{2}H₃] = 0.031) and was stable to extraction. The concentration of vitamin D₃ in pooled plasma collected from 8 healthy adults in late summer was measured as 5.4 ng/ml, a value within the normal range determined by Seemark et al. (5.1 ± 4.8 ng/ml, J. Steroid Biochem. 13: 1057, 1980) using a GC-MS method with vitamin D₃ as internal standard. Our method represents the first stable isotope dilution assay for vitamin D₃ in human plasma. It should be possible to apply a similar hydrogen-deuterium exchange procedure to prepare high isotopic purity trideuterated analogues of the more biologically active 25-hydroxy and 1,25-dihydroxy metabolites of vitamin D₃ for use in stable isotope dilution GC-MS assays for these compounds.
APPLICATION OF FAST ATOM BOMBARDMENT MASS SPECTROMETRY TO BIOCHEMICAL STUDIES OF SECOND MESSENGERS: II, INOSITOL PHOSPHATES

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INTRODUCTION

In a wide variety of cell types inositol-1,4,5-trisphosphate (Ins(1,4,5)P₃), generated by receptor-activated hydrolysis of membrane bound phosphatidylinositol-4,5-bisphosphate, causes rapid release of calcium from internal stores in a signal transduction mechanism regulating diverse cellular activities (1). Subsequent agonist-sensitive Ins(1,4,5)P₃ metabolism involves both further phosphorylation and an inositol salvage pathway catalysed by phosphatase systems, the specificities of which result in the formation of isomeric inositol mono-, bis- and trisphosphates. (1,2) Eukaryotic cells thus contain a large total number of inositol phosphates, exemplified by tumour cells which were recently shown to contain thirteen inositol polyphosphates (3). Identification of inositol phosphates in cell extracts, in particular isomer composition, is central to many metabolic and clinical studies and is currently routinely based on anion exchange HPLC; only rarely is more direct proof of structure obtained via the established four-step sequence of periodate oxidation, borohydride reduction, dephosphorylation and chromatographic/enzymic analysis of the resulting polyols (4,5). Fast atom bombardment (FAB) allows mass spectrometric analysis of inositol polyphosphates without derivatization (6), and, in tandem with collision-induced dissociation mass-analysed ion kinetic energy spectrometry (C1D/MIKES), enables positional isomers of other classes of highly polar, involatile compounds eg the 2',3' and 3',5' isomers of adenosine cyclic monophosphate to be unambiguously distinguished (7). As part of our assessment of the value of mass spectrometric techniques for the analysis of inositol phosphates, we have carried out FAB-CID/MIKES on representative inositol mono-, bis- and trisphosphates.

EXPERIMENTAL

Cyclohexylammonium salts of inositol-1-phosphate and inositol-2-phosphate were obtained from Sigma Chemical Company Limited and the ammonium salts of the 1,4-, 2,4- and 4,5- isomers of inositol bisphosphate and the 1,4,5- and 2,4,5- isomers of inositol trisphosphates were supplied by Boehringer Corporation Limited. Positive ion FAB mass spectra were determined on VG MS 70/70 and ZAB-2F instruments using xenon (8 keV) atoms in the primary beam. FAB-CID/MIKES spectra were obtained on the latter instrument using N₂ as collision gas in the second field-free region, and scanning the electric sector under data system control.

RESULTS AND DISCUSSION

Evaluation of alternative matrix systems, including glycerol, thiglycerol, triethanolamine and polyethylene glycol, together with their aqueous admixtures, established that thiglycerol-based systems gave optimal signal-to-noise ratios in the positive ion FAB mass spectra of the inositol phosphates, typically some three times that obtained with glycerol matrices. Comparison of the sensitivity in the positive ion mode with that in the negative ion mode showed sample derived high mass peaks had absolute intensities 2-3 times those of the corresponding ions observed in the negative ion mode. A small but significant (1-2% relative abundance) peak, corresponding to the protonated molecular ion of the free acid form [MH⁺], was observed in the FAB mass spectrum at m/z 261 (inositol bisphosphate) and m/z 341 (inositol trisphosphates), and these ions were selected with the magnetic sector for CID/MIKES.

For inositol monophosphates characteristic CID-MIKE spectra were obtained from 2 nmol of sample. They were dominated by an intense peak at m/z 99 [H₂PO₄]⁺, which was accompanied by strong peaks at m/z 243 ([MH-H₂O]⁺) and m/z 163 ([MH-H₂PO₄]⁺), together with a series of peaks separated by 18 mass units representing further loss of water at m/z 145 ([MH-H₂PO₄-H₂O]⁺), m/z 127 ([MH-H₂PO₄-2H₂O]⁺) and m/z 109 ([MH-H₂PO₄-3H₂O]⁺). The CID-MIKE spectra of inositol-1-P and inositol-2-phosphate were similar and could not be distinguished either by the presence of isomer specific peaks or comparison of the relative intensity of peaks common to both spectra.

The CID-MIKE spectra obtained from m/z 341 in the FAB mass spectra of the three inositol bisphosphate isomers (25 nmol) each contained peaks at m/z 323 ([MH-H₂O]⁺), m/z 243 ([MH-H₂PO₄]⁺) and m/z 109 ([MH-2H₂PO₄-2H₂O]⁺). However, in the spectrum of inositol-4,5-bisphosphate (Fig. 1(A)) there were prominent ions at m/z 179 and 197 which were absent from the spectra of inositol-1,4-bisphosphate (Fig. 1(B)) and inositol-2,4-bisphosphate (data not shown), which are
Fig. 1. CID/MIKE spectra of m/z 341 generated by FAB-MS of Inositol-4,5-bisphosphate (A) and Inositol-1,4-bisphosphate (B). The m/z values of significant peaks are indicated.

provisionally assigned to rearrangement ions originating from the vicinal phosphate groups (m/z 179 [H₂P₂O₇]⁺, m/z 197 [H₃P₄O₉]⁺). The presence of these peaks only in the spectrum of inositol-4,5-bisphosphate indicates that they may represent a valuable diagnostic indicator for identification of vicinally substituted Inositol polyphosphates.

With inositol-1,4,5-trisphosphate and inositol-2,4,5-trisphosphate determination of characteristic CID-MIKE spectra required a minimum of 38 nmol of sample. The spectra, generated from m/z 421 in the FAB mass spectrum, were dominated by the ion at m/z 403 ([MH-H₂O]⁺), all other ions in the spectrum having relative abundances below 7%. For both isomers, these included m/z 323 ([MH-H₃PO₄]⁺), m/z 305 ([MH-H₂PO₄-H₂O]⁺), m/z 109 ([MH-3H₂PO₄-H₂O]⁺) and m/z 99 ([H₂PO₄]⁺), and in addition, the spectra of both isomers contained a peak at m/z 179, supporting the view that a peak at this mass is characteristic of the CID-MIKE spectra of vicinally substituted inositol polyphosphates.

ACKNOWLEDGEMENTS

We gratefully acknowledge support from the Wellcome Trust.

REFERENCES

The 39th ASMS Conference on Mass Spectrometry and Allied Topics

NITROGEN-CONTAINING SCHIFF BASE DERIVATIVES FOR THE STRUCTURAL DETERMINATION OF LONG-CHAIN ALDEHYDES BY GC/MS.

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The mass spectra of long-chain aliphatic aldehydes and their simple derivatives such as the alkyl oximes do not, in general, contain abundant ions conveying structural information on the alkyl chain. This paper describes the use of Schiff base derivatives containing pyridine or pyrimidine groups for inducing the formation of abundant diagnostic ions containing this structural information.

The aldehyde (0.01 to 0.1 µg) was dissolved in acetonitrile and heated for 15 min with 10 times its weight of 3-(aminomethyl)pyridine, 3-aminopyridine or 2-aminopyrimidine. Aliquots of the reaction mixtures were injected directly onto the GLC column. GC/MS data were recorded with a VG 70/70F mass spectrometer interfaced to a Varian 2440 gas chromatograph which was fitted with a 15 m X 0.2 mm OV-1 fused silica capillary column. The column was operated in the temperature range 200 - 300°C and spectra were recorded at both 25 and 70 eV.

All three types of derivative were produced in quantitative yield and gave a single GLC peak with little tailing. The spectra contained abundant molecular ions and a series of fragment ions produced by cleavage of each carbon-carbon bond of the aliphatic chain. These were formed by a radical-induced cleavage induced by hydrogen abstraction by the heterocyclic nitrogen-containing group (Scheme 1) in the manner previously reported for picolinyl esters of fatty acids and nicotinates of alcohols (1). Although of somewhat lower relative abundance than the comparable ions in the spectra of the pyridine-derived derivatives of fatty acids and alcohols, these ions carried the required structural information.

Scheme 1

The 3-aminopyridine and 3-(aminomethyl)pyridine derivatives of straight-chain, saturated aldehydes produced the diagnostic ions in comparable relative abundance at 14 mass unit intervals as illustrated in Fig. 1 for the 3-aminopyridine derivative.

Fig. 1. Mass spectrum of the 3-aminopyridine derivative of octadecanal.

Aldehydes containing methyl branching produced the characteristic pattern of ions shown in Fig. 2 where the position of the methyl group was indicated by the absence of one of the diagnostic ions (m/z 343 in Fig. 2).
Unsaturation was indicated by both a reduction in spacing of the ions adjacent to the position of unsaturation [40 rather than 42 mass units between the ions at m/z 245 (d) and 285 (f), Fig. 3] and by the appearance of ions of increased relative abundance produced as the result of the abstraction of the allylic hydrogen atoms (ions g and h, Fig. 3). The two abundant ions at m/z 120 and 133 in the spectra shown above can be rationalised by the mechanisms shown in Schemes 2 and 3 respectively.

In general, the 3-aminopyridine derivatives gave slightly simpler spectra than the 3-(aminomethyl)pyridine Schiff bases and both pyridine-containing derivatives gave more abundant diagnostic ions than the Schiff bases prepared from 2-aminopyrimidine.

1) D.J. Harvey, Spectrosc. Int. J., 1990, 8, 211.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

1,1,1,7,7,7-HEXAFLUOROHEPTAN-4-ONE: A NEW DERIVATIZATION REAGENT FOR THE GC/MS OF DIOLS

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Introduction
The use of cyclic derivatives of diols or amino alcohols has been investigated to enhance the selectivity of the derivatization processes, improve chromatographic properties, or direct mass spectrometric fragmentation. We previously employed hexafluoroacetone to form cyclic ketals of diols, but found that harsh reaction conditions were required to effect ring closure (1). The reaction conditions were incompatible with many of the compounds which we wanted to derivatize, and consequently we have sought an alternative reagent. The strong inductive effect of fluorine in hexafluoroacetone influences the cyclization reaction pathway, so that in contrast to acetone, an oxygen atom from the diol leaves during the cyclization, as shown below.

We reasoned that displacing the electron withdrawing CF₃ function from the ketone by one or more -CH₂- groups would alter the reaction characteristics of 1,1,1,7,7,7-hexafluoroheptan-4-one (HFH), and thus restore the facile cyclization chemistry characteristic of acetone. Further, it was anticipated that HFH-ketals might have desirable electron capture properties for negative chemical ionization analyses.

Experimental
The preparation of HFH was similar to that reported by Carpenter et al. (2). Briefly, the magnesium Grignard reagent of 1-chloro-3,3,3-trifluoropropane was condensed with ethylformate, and the resulting alcohol (1,1,1,7,7,7-hexafluoroheptan-4-yl) purified as its p-nitrobenzoate salt (m.p. 73-74 °C). The hydrolyzed alcohol was then oxidized at 0°C using Jones reagent to the product HFH, which was purified by distillation (b.p. 60-61°C/28 mm) prior to use. The mass spectrum of HFH matched that published (2), dominated by the bp at m/z125.

Several published methods for cyclization of ketals were tested. Copper sulfate, trifluoroacetic acid, sulfuric acid, p-toluenesulfonic (TsO') acid, and TsO' pyridinium salt were among the catalysts tested with acetonitrile and/or toluene as solvents, and temperatures varying from 25 to 115°C. The most satisfactory conditions produced the results shown below in Figure 2. Cis-1,2-cyclohexanediol (0.0086mM, 1 mg/mL) in acetonitrile was added to HFH (0.009mM, 2 mg/mL) in acetonitrile in a 13 mm glass tube, and 0.2 mg of TsO' pyridinium salt in 0.1 mL acetonitrile added as catalyst. The tube was heated to 110°C on a hot block in a chemical hood, and fresh acetonitrile added to replace the solvent which evaporated (reaction was never allowed to go dry). The reaction was sampled after 4 hours, and again after 7 hours, adjusting the volume to 1 mL.

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Analytical Conditions
Spectra were obtained on either a HP-5970 mass selective detector, or a Finnigan TSQ - 70 operating in single quad mode, scanning Q3. For chloride attachment experiments, methylene chloride was introduced into the source via a leak valve connected to a Vacumetrics probe. An ion abundance of 500,000 at m/z 35 was used to set the methylene chloride valve. GC was performed using a J&W Scientific DB-225, 30 m x 0.25 mm x 0.15 μm film thickness column on the HP-5980; and an HP Ultra-1, 12 m x 0.20 mm x 0.33 μm film thickness column on the Varian 3400 GC on the TSQ-70. GC conditions were similar on both instruments: 40°C to 200°C at 10°/min., with injection port: at 220°C, transfer line at 200°C.

Results
Reaction products were consistent with the expected in that HFH ketals were formed of both propylene glycol and cis-1,2-cyclohexanediol. The total ion chromatogram and mass spectrum below are typical of these reaction products.

Conclusions
HFH ketals of simple diols can be formed using similar reaction conditions to those traditionally used for acetonide derivatives; however, somewhat more vigorous temperatures, drying or catalysis may be required. The HFH ketals exhibit good chromatographic properties and the expected electron impact fragmentation characteristics. The HFH ketals show a marked temperature dependence with regard to electron capture and chloride attachment in negative chemical ionization. Although the HFH ketals do not exhibit substantial sensitivity in negative chemical ionization, they do show potential as derivatives for positive chemical ionization or electron impact studies of diols.

References
TRACE QUANTITATION OF SCHIFF BASE-BOUND 4-HYDROXY-2-NONENAL AS ITS OXIME, BIS-T-BUTYLDIMETHYLSILYL DERIVATIVE USING 3-HYDROXYNONANAL AS AN INTERNAL STANDARD

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4-hydroxy-2-nonenal (4HNE) is emerging as a biologically relevant by-product of lipid peroxidation. 4HNE has been shown to inhibit glucose-6-phosphatase, to be toxic to cultured cells and to be able to modify low density lipoprotein (LDL) in a manner which promotes atherosclerosis. The method described here uses a novel analogue of 4HNE, 3-hydroxynonanal (HNA), as an internal standard. In addition, the derivatization scheme results in a derivative in which fragmentation is limited and a large portion of the total ion current is concentrated in a characteristic M-57+ ion.

The metabolism of 4HNE is largely unknown. One reaction of 4HNE is binding to protein through Schiff base-linkages. This reaction would not be expected to be enzyme catalyzed but the large number of free amine residues available on proteins in biological material could make this an important pathway. Quantitation of 4HNE in either its free form or bound to protein through Schiff base-linkages is achieved by forming an oxime derivative of the aldehyde in a nucleophilic substitution reaction. The bis-t- butyldimethylsilyl derivative is then formed prior to analysis by selected ion monitoring (SIM) gas chromatography/mass spectrometry.

Experimental. All solvents and reagents are purchased from commercial sources. 4HNE is synthesized according to the method of Esterbauer. HNA is synthesized by the reaction of mercuric acetate with 2-nonenal.

Sample preparation. Several types of samples have been analyzed including cultured cells, tissues, whole blood and plasma. For the analysis of cultured cells, the media is poured off, the cells harvested, lysed in 2 mL of deionized water and derivatized as described below. 1-50 mg tissue samples are homogenized directly in the oximation reagent described below. 1.0 mL aliquots of whole blood or plasma are analyzed directly under the reaction conditions described below. In each case, an appropriate amount of HNA in water is added as an internal standard immediately before beginning the oximation reaction.

The oximation is carried out in 4 mL of 0.3 M hydroxylamine hydrochloride in a 0.1 M acetate buffer, pH 5 with 50 µM butylated hydroxytoluene an 1 mM DETAPAC. The samples are thoroughly mixed and reacted for 1 hour at 70°C. The lipid aldehyde-oximes are recovered by octadecylsilyl solid phase extraction and eluted in methanol. The methanol is evaporated and the residue reconstituted in 50 uL of MTBSTFA and 200 uL dimethylformamide and reacted at 70°C for 1 hour. The derivatives are extracted into petroleum ether, evaporated to dryness and reconstituted in 50 uL of isooctane for analysis.

Gas chromatography/mass spectrometry. A double focusing, reverse-geometry mass spectrometer (Model 8230, Finnigan-MAT) is used. The instrument is equipped with a Varian 3700 gas chromatograph with a 15 m x 0.32 mm capillary column with a 0.25 µm film thickness bonded phase polydimethylsiloxane stationary phase (SE-30 Econoscap, Alltech Associates). Injections are made on-column and the gas chromatograph programmed from 100°C to 290°C at 15°C/minute. The mass spectrometer is operated with 70 eV electron ionization in the selected ion monitoring mode at a mass resolution of 2500 monitoring m/z 342.2 (4HNE, M-57+), m/z 344.2 (HNA, M-57+) and using 342.9793 of the reference compound perfluorokerosene as a lock mass. Quantitation is achieved by integrating the appropriate chromatographic peak areas for 4HNE and HNA. The area ratio of 4HNE/HNA is converted to pmoles of 4HNE using a calibration curve determined from standard amounts of 4HNE and measured in the same experiment.

Results and Discussion. The electron ionization mass spectra of the oxime, t-butyldimethylsilyl derivatives of 4HNE and HNA are shown in Figure 1. As can be seen, an ion of high relative intensity is observed for each compound which corresponds to the loss of C₄H₉ from the respective molecular ions.
Calibration curves have been found to be linear from 0 pmoles to > 700 pmoles. In these experiments a limit-of-detection of approximately 1 pmoles (156 pg) has been found. This limit-of-detection is sufficient for the analysis of the sample types encountered in our investigations.

Typical chromatograms are shown in Figure 2 for the analysis of cultured cells and human plasma. These samples contain 29 and 18 pmoles of 4HNE, respectively. An excellent signal-to-noise ratio is observed and few additional peaks are observed in this time window. The doublets formed in the oximation reaction are typical for this nucleophilic substitution reaction.

An important concern in these experiments is the possibility of auto-oxidation of the sampler and the artifactual formation of 4HNE. We found that the oximation, without the addition of antioxidants (BHT and DETAPAC), does result in the formation of significant amounts of 4HNE. In our hands this amounted to 200 to 400 pmoles of 4HNE formed in cell samples which contained approximately 60 pmoles. The concentrations of BHT and DETAPAC used here were effective in eliminating the formation of 4HNE in even high concentration linoleic acid solutions and does not interfere with the assay.

Precision was evaluated in a number of different ways. Replicate analysis of standards indicate relative standard deviations of 7.4% and 4.1% at 100 pmoles and 500 pmoles levels, respectively. The analysis of a series of paired blood samples indicates an average relative standard deviation of 10.3% in samples ranging from 10 to 30 pmoles/ml. Finally, the analysis of triplicate sets of cultured cell exposed to 95% and 20% oxygen show average relative standard deviations ranging from 7% to 47% with an average of 26% (n=18). Overall, precision consistent with this type of assay was observed.

Accuracy was evaluated by standard addition analysis of human plasma samples. This analysis indicated no significant bias in the assay. The accuracy of the method is aided by the chromophore due to the α,β-unsaturated carbonyl. This chromophore allows the preparation of dilute solutions of accurately known concentration. Finally, the use of the methoxime analogue of 4HNE for the preparation of standards allows the Schiff base-to-oxime reaction to be more closely modeled and further enhances the accuracy of the method.
Mass Spectrometry of Some Novel Amino β-Lactam Carboxylic Acids (ABCs).
New Matrices and Exact Mass Analyses.

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FABMS analysis of some zwitter-ionic β-lactams has given poor signal-to-noise (s/n) values in treitol/erythritol, glycerol, and/or thioglycerol. Ion-pairing the analyte provided a way to improve the value of s/n from ~1 to ~20% of base peak (1). Ion-pairing as a technique for increasing ion-emission by FAB has been reviewed (2). It remains difficult to analyze the numerous β-lactams we produce by our four step, Leuchs anhydride based, synthetic strategy (3). In this report we describe three MS based strategies used to provide mass spectrometric data: 1) derivatization to afford exact mass data; 2) an acidic matrix with useful surface concentrations of the zwitter-ionic analyte; and 3) the use of an ancillary reagent to enhance analyte ion intensity.

The derivatization proved viable as illustrated by compounds I and II. These novel biologically active monobactams I and II were synthesized through the Leuchs anhydride strategy (3,4). After methylation both yielded confirmatory exact mass data:

<table>
<thead>
<tr>
<th>Monobactam</th>
<th>Ion</th>
<th>Calc</th>
<th>Found</th>
<th>Error</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>[M-1]+</td>
<td>229.1188</td>
<td>229.1162</td>
<td>2.2 mmu</td>
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<td>4.4</td>
</tr>
</tbody>
</table>

Other zwitter-ionic monobactams gave useful information by this strategy. Benzoylation and methylation was combined to give a derivative useful for LC clean-up as well as characteristic useful fragmentation patterns. 6-Aminopenicillanic acid illustrates this:
The indicated $M^+$ (m/z 334) and cleavage products were readily confirmed by exact mass EIMS. $\beta$-Benzyolamidopenicillanic acid methyl ester was generated as a reference compound for our characterization of the ABCs. All of the cleavages typical of penicillins shown here were confirmed by EIMS exact mass. The importance of benzoylated ABC methyl esters will be their relative ease of clean-up by C18 RP HPLC. The ABCs typically are produced as a mixture of four diastereomers which must be separated for structural assignment and characterization for antibiotic activity.

FABMS of these labile zwitterionic ABCs was facilitated by the use of pyruvic acid as a matrix. As a matrix it shows only ions of low mass. The base peak was m/z 143; no identifiable monomer (m/z 176), dimer, trimer, ... peaks were seen above background. Peaks as yet not identified provide a low background up to $\sim 700$ m/z. Pyruvic acid is a good solvent for peptides and proteins as well as $\beta$-lactams. Unlike glycerol where a surface excess for APA is not found (1), a signal $\sim 12$% of the base peak is seen for MH$^+$. It is possible that the pyruvic acid preparations have a tendency to form reverse micelles. Outside of the as yet unknown micelle concentration range, the more polar compounds give a useful surface concentration if not a surface excess.

Enhancement of FAB ion yield was achieved by using an ancillary reagent. It was felt that if a component of the matrix breaks down to gaseous products by the action of the high energy atomic beam, then a substantial increase in gas phase ion yield should result. The reagent showing the desired effect was tetrazole. Tetrazole is known to decompose to gaseous products when heated:

$$\text{NCH}_2\text{N} \stackrel{\Delta}{\rightarrow} 2\text{N}_2 + [\text{CH}_2]$$

To be useful, the ancillary reagent concentration must not be high enough to allow an uncontrolled chain reaction to be triggered by the atom or ion beam. It has good solubility in pyruvic acid (15-20%) at room temperature. The quasi-molecular ion of the gastrin peptide WMDF-NH$_2$ was enhanced sixfold when tetrazole was in the matrix.

References
Characterization of Reactive and Dissociative Behaviors of Biolactones. Tracy Donovan and Jennifer Brodbelt, University of Texas, Austin, TX 78712-1167.

In this work, we have undertaken an examination of the ion-molecule chemistry of some analytically relevant biolactones: ascorbic acid, several gluconolactones, and a pyrrole lactone (dimethylpyrrole-$\gamma$-butyrolactone) which is a potent plant growth regulator (see Figure 1). The reactive and dissociative behaviors of these biolactones were studied using a quadrupole ion trap mass spectrometer and a triple quadrupole mass spectrometer, and compared to simple lactones.

All three biolactones studied in the quadrupole ion trap (D-gluconolactone, L-glucono-1,5-lactone, and the pyrrole lactone) were protonated by dimethyl ether ions, but none formed methyl addition products that were commonly formed in the earlier study of simple lactones. Rather, each of the biolactones formed a methylene substitution product, (M+13)$^+$. In the case of the pyrrole lactone, the intensity of the (M+13)$^+$ adduct was nearly fifty percent higher than the intensity of the protonated pyrrole lactone. Methylene substitution adducts of the two gluconolactones were roughly one quarter the abundance of their corresponding protonated molecules.

In order to determine the site of methylene substitution, dimethyl ether ion reactions with simpler compounds representative of small portions of the biolactones were compared. For example, upon dimethyl ether chemical ionization, dimethyl pyrrole forms an (M+13)$^+$ adduct, but $\gamma$-butyrolactone does not. Therefore, methylene substitution of the pyrrole lactone presumably occurs at the pyrrole ring rather than at the lactone ring.

A comparison of the CAD spectra of protonated pyrrole lactone and its (M+13)$^+$ adduct, shown in Figure 2, reveals similar structural information about the adduct. The ion at m/z 94 in the spectrum of protonated pyrrole lactone, presumably due to the cleaved pyrrole ring, shifts 13 amu to m/z 107 in the CAD spectrum of (M+13)$^+$. This gives additional support to the proposal that the site of methylene substitution is the pyrrole ring rather than the lactone ring.

A mechanism which has been elucidated for (M+13)$^+$ formation with substituted aromatic substrates may also be responsible for (M+13)$^+$ formation from the pyrrole lactone:

![Reaction Diagram]

The reaction is initiated by nucleophilic attack on the dimethyl ether cation by the double bond, and is followed by elimination of methanol. The adduct structure has a high degree of resonance stabilization, which may explain the greater abundance of this ion relative to the protonated pyrrole lactone.

Upon collisional activation of all three protonated biolactones, the predominant dissociative pathway is dehydration followed by decarbonylation (loss of 46). A possible mechanism which parallels the dissociative routes of simple protonated lactones is shown in Scheme 1. Additional dissociative pathways
include decarbonylation and decarbonylation combined with loss of multiple water molecules. Pathways such as neutral alkene or ketene loss which are important dissociative pathways in less complex protonated lactones are not observed in the fragmentations of the protonated biolactones.

The reactive behavior of protonated biolactones with neutral reactive gases was investigated in a triple quadrupole mass spectrometer. After FAB ionization and mass-selection by the first quadrupole of a triple quadrupole mass spectrometer, each protonated biolactone was allowed to undergo reactive collisions with selected molecules in the collision quadrupole. The protonated biolactones were completely unreactive with ethylene in the collision quadrupole, but they formed abundant proton bound dimers with dimethyl ether and ethylene oxide at reactive gas pressures above 2 mtorr and collision energies between >0 and 8 eV.

Figure 1. Biolactone Structures

![Biolactone Structures](image)

Figure 2. CAD Spectra of Pyrrole Lactone

Scheme 1. Dehydration/Decarbonylation of Protonated Pyrrole Lactone

![Scheme 1](image)
Mass Spectrometric Validation of Cadaverine and Putrescine in Fish.

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²: Fishery Research Branch, Food and Drug Administration, P.O.Box 158, Dauphin Island, Alabama 36528

Cadaverine and putrescine occur naturally in food of animal origin as the results of microbial spoilage. These diamines (C₄ and C₅ alfa,omega-diamines) are good indicators of the food-decomposition in fishery products for human consumption.

The presently used analytical method is J.A.O.A.C. 64, 584, 1981. This is a gas chromatographic (GC) procedure carried out with the pentafluoropropionyl derivative of the purified and dried fish-extract. 1,6-diaminohexane is used as internal standard, and nitrogen-specific or electron-capture detector is recommended to achieve adequate sensitivity together with some degree of specificity. The method, however, has never been verified by mass spectrometry.

We carried out the analysis on several fish samples using a hybrid-tandem instrument as a mass specific detector in capillary GC work and verified the results obtained by an ordinary GC with packed column and nitrogen specific detector. Improperly handled salted, smoked fish samples had putrescine levels of up to 233 µg/g (vs 2.1 for fresh controls) and up to 2346 µg/g (vs 3.2 for fresh controls) of cadaverine.

Electron ionization (EI), positive chemical ionization (PCI) and electron capture CI (EC-CI) was used in these measurements. The spectra, especially the CI spectra showed some interesting characteristics. The methane PCI spectra consisted of two peaks: a base peak representing the molecular cation (MH⁺) and a minor one formed by the loss of a pentafluoropropionamide molecule. The negative spectra consisted of several peaks, and the changes of the fragmentation patterns between the homologous diamines were surprisingly unpredictable. The total ion current was 100 times larger in the negative spectra than in the positive spectra, and the intensity ratios between the dominant negative and the positive ion-species were about 40.

In addition to the pentafluoropropionyl derivatives, dimethylaminomethylene derivatives were prepared using dimethylformamide dimethylacetal reagent. These derivatives formed even easier than the fluoroacetyl derivatives and exhibited excellent chromatographic and mass spectral characteristics both for GC/MS and GC/MS/MS analyses.
Pentafluoropropionyl derivatives

Dimethylaminomethylene derivs

CI⁻ spectra

CI⁺ spectra
DETECTION AND IDENTIFICATION OF COMPOUNDS RELATED TO OKADAIC ACID USING LC/MS AND TANDEM MS

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Okadaic acid, a diarrhetic shellfish poisoning (DSP) toxin, has been identified in several dinoflagellate species, including Prorocentrum concavum¹. Illness attributed to DSP is due to the consumption of shellfish contaminated with these dinoflagellates.

The development of instrumental methods for DSP toxins has been a challenge due to their low concentration in complex matrices, structural complexity, and high molecular weight (Table 1). The development of any analytical method requires standards. Okadaic acid (OKA) is currently the only DSP toxin that is available commercially and it is extremely expensive. We chose to culture the dinoflagellate, P. concavum, in our laboratory as an alternate source of OKA.

The most widely-used instrumental method is reversed phase HPLC with fluorescence detection (FLD). Fluorescent derivatives of OKA are synthesized by esterification of the carboxyl group using derivatizing reagents such as 9-anthracenyl diazomethane (ADAM)². Using this method, confirmation of peak identity is not possible. An ion-spray HPLC-MS method has been developed for the confirmation of OKA, and for the detection of related compounds in marine samples³.

OKA is isolated from the cultured dinoflagellates using a sequential chromatography column method developed in our laboratory. To date, hundreds of milligrams of pure OKA have been isolated and characterized using several NMR and MS techniques.

Recently, other compounds, related to OKA, have been detected in the P. concavum culture. Isolation and subsequent analyses of these compounds indicated that they are the methyl ester and two diol ester derivatives of OKA. Other diol esters have been identified in cultures of P. lima², but not in P. concavum. These compounds are not naturally-fluorescent and would not be detected using the conventional HPLC-FLD method, since the carboxyl functionality is not available for derivatization. Ion-spray HPLC-MS was used for the initial screening of the culture samples containing these compounds (Figure 2). Positive ion LSIMS (Figure 3) and NMR were used to aid in the identification of these natural derivatives.

Ion-spray LC-MS has proven to be a powerful method for screening samples for DSP toxins and related compounds. We are currently using this technique to screen other strains of Prorocentrum and other samples, including shellfish tissue, for new DSP toxins. Utilization of ion-spray LC-MS/MS to aid in the characterization of these compounds has begun.

References:
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

FIGURE 1: Ion-spray HPLC-MS analysis of a fraction obtained during the clean-up procedure of the cultured dinoflagellate, *P. concavum*, using a 250 x 2.1 mm I.D. Vydac 201TP52 column with a 200 µL/min linear gradient elution (40-100% acetonitrile plus 0.1% trifluoroacetic acid in 15 minutes). This fraction contains OKA and the ester derivatives.

**Table 1: Structures of DSP toxins and related compounds.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKA</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Dinophysistoxin-1 (DTX-1)</td>
<td>H</td>
<td>H</td>
<td>Cl₂</td>
</tr>
<tr>
<td>Dinophysistoxin-2 (DTX-3)</td>
<td>H</td>
<td></td>
<td>acyl Cl₂</td>
</tr>
</tbody>
</table>

**FIGURE 2: The positive ion LSIMS mass spectra of the a) OKA diol ester (MW=914); b) OKA methyl ester; and c) OKA diol ester (MW=928) isolated from the cultured dinoflagellate, *P. concavum*.**
ANALYSIS OF MICROORGANISMS BY QUARTZ TUBE PYROLYSIS GAS CHROMATOGRAPHY MASS SPECTROMETRY

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The detection and identification of microorganisms is of significant interest in numerous scientific areas. Of the myriad of chemical compounds that can be found in microorganisms the determination of their lipid composition has been suggested to be closest to an ideal chemotaxonomic method, and is the basis of the fatty acid methyl ester (FAME) approach. The utility of this approach has been extended by the recent demonstration that bacterial species could be successfully differentiated by analysis of their underivatised lipid components using a Curie point pyrolysis gas chromatography ion trap mass spectrometry (Py-GC-MS) system(1). Quartz tube pyrolysis offers several advantages over Curie point pyrolysis, both in terms of sample preparation and control over pyrolysis conditions. A quartz tube Py-GC-MS system was constructed and evaluated by investigating three bacterial species: Bacillus anthracis, Bacillus subtilis, and Staphylococcus albus.

The lipid compositions of the three bacterial species could be easily distinguished by a visual inspection of the high boiling point region (300-450s) of the total ion chromatograms (Figure 1.). The signal averaged mass spectra for this region are also reproduced in Figure 1. The spectra are characterised by a series of ions differing by consecutive 14 amu increments, which is consistent with the assignment that this region comprises the lipid components of the bacteria. The three bacterial species could be readily distinguished by differences in the relative intensities of the ions at m/z 522, 550 and 578 in the averaged spectra.
Figure 1. Total ion chromatograms and signal averaged mass spectra for *Bacillus anthracis* (BA), *Bacillus subtilis* (BS) *Staphylococcus albus* (SA).

References:

There is widespread interest in the structure elucidation of isolated natural products because of the diversity of biologically active compounds that are produced by plants and micro-organisms. The structure elucidation of complex natural products at Bristol-Myers Squibb typically involves the use of gentle ionization techniques (FAB or thermospray) for the generation of molecular ion species and fragment or substructure ions in the gas phase. Through the coordinated use of tandem mass spectrometry and high resolution mass spectrometry, these substructures are rapidly characterized and identified. This strategy results in a more rapid, streamlined structure elucidation process.

We have recently employed both chemical and enzymatic microscale reactions on the FAB probe tip to further characterize and identify unknown isolated natural products. This allows for the selection of a specific chemical or biochemical reaction to produce substructures that may not be formed in the gas phase. This strategy provides an alternate method of substructure analysis that is complementary to MS/MS analysis. Furthermore, the specificity of this method allows for the direct evaluation of chemical reactivity, determination of amino acid sequence, and identification of targeted functional groups.

This methodology requires small quantities of sample with minimal sample handling. Reaction mixture can be directly analyzed by FAB/MS with no reaction work-up. The coupling of our existing structure elucidation protocols with this novel methodology provides further insights into the molecular structure of unknown natural products.

Here we demonstrate that microscale chemical and enzymatic reactions with isolated natural products can be performed on probe and directly monitored and analyzed by FAB/MS. This methodology is illustrated with amino-aveninomycin D, aveninomycin D, esperamin A, and substance P. All reactions were performed on the FAB probe tip and directly analyzed by FAB/MS on a Kratos MS50 mass spectrometer. Aveninomycin D (2 µg), aveninomycin D (2 µg), and esperamin A (0.2 µg) were dissolved in 2 µl of m-nitrobenzyl alcohol, and treated with 0.1 µl of acetic acid for three hours (aveninomycins) or 0.2 µl of thioglycerol for 20 minutes (esperamin A). The substance P digest with carboxypeptidase Y was carried out with 2 µl of 50 mM ammonium acetate (pH 6), 1 µg of substance P, and 0.01 units of carboxypeptidase Y. The reaction was stopped by the addition of 1 µl of m-nitrobenzyl alcohol.

The full scan negative ion FAB mass spectrum of aminoverninomycin D (MW 1505) contains ions that correspond to the losses of successive sugar substructures (m/z 1158, 968, 808, 664, and 534). To determine the location of the ortho-esters, aminoverninomycin D and an everninomycin D standard (MW 1535) were exposed to hydrolysis conditions on probe and analyzed by FAB/MS. The resulting mass spectra exhibit two additional protonated molecular ions 18 and 36 amu higher, corresponding to the addition of a water molecule to each ortho-ester (Figure 1). By comparing which fragment ions were shifted by 18 and 36 amu, the location of the ortho esters of aminoverninomycin D were determined to be sugars three and seven.

Treatment of esperamin A (MW 1324) with thioglycerol reduces the trisulfide and triggers the cyclization of the diones moiety.2 The mass spectrum of this microscale reaction mixture (MW 1248) contains new and more abundant fragment (substructure) ions than that of the parent compound. Furthermore, by noting those ions that are shifted by 76 amu, the altered substructure is readily indicated. Analysis of this product by our coordinated approach with MS/MS and HRMS would result in complementary substructure information and aid the structure elucidation process if this were an unknown.

To demonstrate that the selectivity and mild reaction conditions associated with enzymatic reactions can be utilized with this methodology, the carboxypeptidase Y digest of substance P was performed on probe. Carboxypeptidase Y successively cleaves the C-terminal amino acid of a peptide/protein, so the sequence from the C-terminus can be determined by monitoring the reaction.4,5 From the FAB mass spectra of this reaction performed on probe, the sequence of five successive amino acid residues from the C-terminus is readily indicated (Figure 2).

References
Figure 1. Negative Ion FAB Mass Spectra of the Probe Hydrolysis Products of Everninomycin D (MW 1535) and Amino-Everninomycin D (MW 1505).

Figure 2. FAB Mass Spectra of the Carboxypeptidase Digest of Substance P on Probe.
Coordinated Use of Tandem Mass Spectrometry and High Resolution Mass Spectrometry for the Rapid Structure Elucidation of Isolated Natural Products

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Many of the current therapies for the treatment of cancer and infectious diseases are developed from isolated natural products. These compounds are components of complex mixtures isolated from micro-organism fermentation broths or extracts of plant material. These sources often yield novel classes of compounds that possess a unique mechanism of action or spectrum of activity. Since natural products are a source of truly novel lead compounds, Bristol-Myers Squibb has an active world-wide research effort dedicated to the rapid discovery and identification of novel natural products.

A structure elucidation strategy involving the coordinated use of tandem mass spectrometry (MS/MS) and high resolution mass spectrometry (HRMS) is employed to provide rapid and accurate insights into the various substructures of an unknown molecule. The premise of the method is based on the fact that fragment or substructure ions observed in daughter MS/MS spectra are typically observed in full scan mass spectra. Our approach involves the daughter MS/MS mode to divide a complex molecule into smaller and perhaps simpler substructure molecules. The parent MS/MS mode is utilized to determine "mass spectral connectivity" and link the related substructures. The utilization of the parent MS/MS mode to provide substructural connectivity is analogous to a retro-synthesis in the gas phase. Once related substructures have been identified, accurate mass measurements and molecular formulas are obtained via high resolution mass spectrometry.

Figure 1 shows the major substructures of Amino-Evennimicin D formed via full scan DCI/MS experiments. Further detailed substructural information was obtained via the daughter MS/MS spectrum of m/z 666 (Figure 2). Parent MS/MS analysis of the major daughter ions observed in the spectrum established substructural connectivity and the resulting accurate mass measurements were performed. For example, the parent MS/MS spectrum of the m/z 233 substructure contained the m/z 345, 353 and 491 ions establishing connectivity. Accurate mass measurements of these ions resulted in possible molecular formulas of these substructure ions as well as molecular formulas for the resulting neutral loss substructures. However, the parent MS/MS spectrum of the m/z 158 substructure (amino sugar) does not contain the m/z 233 ion indicating that no mass spectral connectivity exists between these two substructures. Thus, the two substructures result from separate and unique fragmentation schemes. No neutral loss information (mass and molecular formula) can be derived from the m/z 158 and 233 substructures of Amino-Evennimicin D.

Figure 1. The Major Substructures of Amino-Evennimicin D Observed Via Methane DCI/MS.

Figure 2. The Daughter MS/MS Spectrum of the m/z 666 Substructure of Amino-Evennimicin D.
A similar methodology was utilized to elucidate substructures of Taxol and Luzopeptin (Figure 3). Figure 4 shows the parent MS/MS spectrum of Taxol selecting the m/z 105 substructure (benzoyl). Structural information corresponding to the taxol side-chain (m/z 286) is present along with an ion corresponding to baccatin substructure (m/z 509). The presence of the m/z 509 ion is due to a separate benzoyl substructure on the baccatin moiety. Figure 5 shows the daughter MS/MS spectrum of the m/z 592 substructure of Luzopeptin. Fragment ions corresponding to the quinoxaline and each amino acid substructures are present. The accurate mass measurements provided molecular formulas of these corresponding substructures.

![Figure 3. Structures of Taxol and Luzopeptin.](image)

![Figure 4. The Parent MS/MS Spectrum of Taxol Selecting for the m/z 105 Benzoyl Substructure (resolution of [M+H]+ adjusted).](image)

![Figure 5. The Daughter MS/MS Spectrum of the m/z 592 Substructure of Luzopeptin.](image)
DETERMINATION OF OLIGOGALACTURONIDES HAVING PHYTOALEXIN ELICITING ACTIVITY IN PLANT CELL CULTURES BY FAB AND IS MASS SPECTROMETRY.

Andrea RAFFAELLI, Sergio PUCCI, Flavia MARINELLI, Plero SALVADORI

Pathogenic fungi and bacteria

Plants respond to phytopathogenic attacks with a complex of pre-formed and inducible defence reactions (Figure 1). Signals able to trigger defence reactions in plant cells are called elicitors. Elicitors can be of abiotic origin, such as agents of physical and chemical stress, or biotic molecules both exogenous or endogenous. The identification of the "chemical signals" responsible of the elicitation process can be extremely useful in the field of biotechnology, in order to force plant cells to produce specific chemicals of potential interest as drugs.

Partial digestion (Figure 2) of polygalacturonic acid in the presence of endopolygalacturonase, isolated from a commercial preparation of Aspergillus niger pectinase, produces mixtures of α-D-1,4-galacturonide oligomers which elicit the phytoalexin metabolism in some plant cell cultures. These oligomer mixtures, obtained at different digestion times (30 min., 2, 4 and 20 hours), were analyzed by FAB mass spectrometry to establish their composition and the nature of the oligomers present.

This method of analysis could be of large interest for the identification and structural determination of oligosaccharides coming from enzymatic degradation of plant cell walls, which is one of the most important points in the investigation on the phytoalexin elicitation.

Figure 1 Schematic representation of the defence reactions of a plant cell against phytopathogenic attacks.

acetic acid and desalted by a cation exchange resin to convert carboxyl groups to their protonated form. These samples were dissolved in a glycerol/thioglycerol matrix and analyzed by a VG 70/70E mass spectrometer in FAB negative ion mode using a xenon gun. The results obtained allow to establish the oligomeric composition of the longest hydrolysis times mixtures, ranging from 2 to 8 depending on the hydrolysis time. The spectrum of the 2 hours mixture, the one containing the highest oligomer detected, is reported in Figure 3. The 30 min mixture behaves differently with respect to the others. The background is very high and it is not possible to estimate the presence of oligomers with a degree of polymerization higher than 6.

This method of analysis could be of large interest for the identification and structural determination of oligosaccharides coming from enzymatic degradation of plant cell walls, which is one of the most important points in the investigation on the phytoalexin elicitation.
The same mixtures were examined also in LC-MS with ionspray (IS) ionization on a NERMAG R3010 triple quadrupole instrument with a custom built ion source. The results obtained are in a very good agreement with those obtained by FAB.

**Polygalacturonic acid (PGA)**

![Polygalacturonic acid (PGA)](image)

Figure 2 Scheme of the partial enzymatic digestion of polygalacturonic acid.

**Figure 3** FAB-MS spectrum (glycerol/thioglycerol) of the 2 hours digestion mixture.
Membrane introduction mass spectrometry has received considerable attention in our laboratory. The work has resulted in the development of a hollow fiber membrane direct insertion probe [1] that is unique in that it locates the membrane in the ion source of the mass spectrometer. This location of the membrane reduces problems due to analyte dilution, memory effects and poor response times observed in interfaces where the membrane is located remote from the ion source. The flow-through design of the direct insertion membrane probe facilitates analyte transfer to the mass spectrometer and allows the use of flow injection analysis sampling methods, thereby greatly facilitating the task of automated, continuous monitoring. Recently a second generation membrane probe has been developed that employs a sheet membrane and temperature control [2]. The direct insertion sheet membrane probe (Figure 1) has been well characterized [2] and then utilized for bioreactor monitoring [3].

A schematic diagram of the flow injection analysis (FIA) instrumentation is given in Figure 2. The membrane probe utilized was a sheet direct insertion membrane probe (Figure 1) when a triple quadrupole instrument [3] was used and a simple capillary membrane probe when an ion trap detector was used [4]. Both membrane probes were held at a constant temperature of 70°C for these experiments. The membrane is a dimethyl vinyl silicone polymer with a thickness of 0.01 in. Plugs (250 µl) of fermentation broth or standard solution are injected into the continuous stream of dilute acid by a Waters Filter Acquisition Module (FAM). All valves are controlled via a flow injection analysis control unit built on-site. A sampling rate of 15 times/hour is possible with this system. Recently a gas mixer, which allows automatic changes in the oxygen concentration in the feed stream, was added to the measurement system. The mass spectrometer, the flow injection analysis unit and the gas mixer can all be controlled by a personal computer, a combination which allows feedback control of bioprocesses.

The fermentations of particular interest are the fermentation of the microorganisms Bacillus polymyxa and Klebsiella oxytoca. Bacillus polymyxa produces high optical purity (R,R)-(−)-2,3-butanediol. Interest in Klebsiella oxytoca is based on the ability to produce 2,3-butanediol almost exclusively in the meso form. These organisms produce carbon dioxide, acetic acid, acetoin, 2,3-butanediol and ethanol as the major products in relative proportions which depend on the availability of oxygen.

On-line quantification of the major solution phase products was done using external standards with the automated FIA method of sampling. These methods provide a sampling rate sufficient to give smooth curves displaying the concentration of the products during a complete fermentation. The concentration profiles of the products, acetoin, ethanol and acetic acid, were derived from the protonated molecules, (M + H)+, appearing at m/z 89, 47 and 61 respectively. For butanediol, the (M + H - H2O)+ ion m/z 73 was monitored since it was far more abundant than the protonated molecule. Measurement of acetic acid required on-line acidification of the sample, since acetic acid is in its dissociated form in the fermentation pH range of 5.7 to 7 and therefore does not pass the membrane. An example of on-line quantitation of the major products is shown in Figure 3. The concentrations measured by on-line membrane introduction mass spectrometry were confirmed by off-line gas chromatography.

In the course of the on-line monitoring experiments, fermentation off-gases and dissolved gases could also be measured [3]. Based on the measured off-gas data and measured concentrations of the major solution phase products, a reaction network was developed which allowed estimation of glucose and biomass concentrations [5]. Excellent estimates of biomass and of glucose concentrations were produced, as compared to experimental measurements, during the course of fermentation [5].

The tandem mass spectrometry capability of a triple quadrupole mass spectrometer is useful in allowing confirmation of product identity and identification of metabolites and intermediates by comparison with MS/MS spectra of authentic compounds [3]. This capability also forms the basis for analysis of optical isomers; for example the R,R and meso forms of 2,3-butanediol were distinguished by recording MS/MS product spectra of phenylboric ester derivatives of these compounds. A large difference in the ratio of the fragments at m/z 133 and 73 for the two derivatives makes isomer distinction possible.
The fully automatic flow injection analysis-membrane introduction mass spectrometry system is capable of on-line feedback control of bioreactors. From fermentations without feedback control it is known that the production rate of acetic acid is related to the availability of oxygen in the fermentation broth. A decrease in acetic acid production rate reflects a decrease in the oxygen availability (large oxygen consumption) and an increased rate of formation of acetol, 2,3-butanediol and ethanol. The amount of 2,3-butanediol might therefore be increased if the oxygen feed were adjusted during the fermentation, while controlling the feed of oxygen on the basis of the rate of acetic acid formation. Figure 4 shows the product distribution for an experiment controlled by this way. After approximately 2 hours, the production rate of acetic acid passed a preset upper limit and the oxygen partial pressure was slowly reduced until a lower limit in the production rate was reached (hour 5). In order to maintain a fast production rate of butanediol the oxygen feed was increased again. From Figure 4 it can be seen that the production rate of butanediol increases again until the oxygen availability becomes too high (hour 6).

References
There are several commercial operations using CYANEX® 272 extractant to selectively recover cobalt from aqueous cobalt/nickel streams. CYANEX 272, a dialkylphosphinic acid, is used in conjunction with a diluent - usually a mixture of C10-C16 hydrocarbons. When the two phases are contacted under slightly acidic condition, the cobalt is quantitatively and selectively extracted into the organic phase (1). However, in the absence of an antioxidant cobalt will catalyze the air oxidation of the diluent to fatty acids. (2) The long chain fatty acids are detrimental in that they build up in the solvent and will extract both cobalt and nickel in a non-selective manner. Rigid control of the fatty acids is critical of the successful operation of the plant.

This paper discusses a rapid GC/MS method to quantify the fatty acids as their methyl esters using a HP 5870 MSD. Normal GC techniques to determine the fatty acids as their methyl esters are not applicable since they coelute with the multitude of varsol components which are 1-2 orders of magnitude higher in concentration.

The esters were detected by operating the HP 5870 in the selective ion mode (SIM) using only the 74.00 amu ion. This ion is characteristically abundant in the spectra of all linear fatty acid methyl esters. It’s not found in the spectra of hydrocarbons or other oxidation products such as alcohols and aldehydes which coelute with the esters.

Fatty acid esters can be detected by extracting the 74.00 amu ion chromatogram obtained from the SCAN mode of operation. However, detection limits are at least an order of magnitude higher and quantitation is poor with only 2-4 data points per peak.

Docosanoic acid was used as an internal standard since only C5 to C18 acids were observed in the matrix. 0.1 - 4.0 g/l C5 to C24 acid standards were prepared using 250 g/l CYANEX 272 extractant in a C10-C16 hydrocarbon diluent as the solvent. The standards were derivatized by heating 400 μl of each standard mixture with 400 μl of 1.57 g/l docosanoic acid and 400 μl of METHYL-8 (4) at 60° C for 45 minutes. The inlet for the MSD was a HP 5890 GC fitted with a 30 m X 0.25 mm DBS column - film thickness 0.25 microns. 0.1 μl were injected at a head pressure of 11 psig and with the vent flow set at 100 cc/min. The injection port was at 300° C while the oven was heated from 50 to 300° C at 10°/min.

The 5970 MSD was operated under SIM conditions using only the 74.00 amu ion at low mass resolution i.e. 0.7 - 0.9 amu. The instrument was manually tuned using ions 219, 131 and 69 from the perfluorotributylamine calibration gas for masses 1, 2 and 3. This optimized the MSD for maximum 74.00 ion sensitivity. The electron multiplier tune value was 1800 V. It was increased to 2000 V for increased sensitivity. Using a dwell time of 50 ms, 10-15 data points were obtained on the smaller peaks.

All calibration curves were linear over the expected operating range of 0-5 g/l. Figure 1 is a typical example. The lower detection limit (S/N = 4) was 0.005-0.01 g/l for most acids. The relative response factors (docosanoic acid = 1) varied from 0.8 to 1.85 with undecanoic acid having the maximum sensitivity (Figure 2). The variation in response factors is a result of two opposing factors: 1) the fraction of 74.00 amu ion content of the total ion current and 2) the disproportionate splitting of the sample. The lower fatty acids have a higher proportion of 74.00 amu ion but at higher injection port temperatures, a greater fraction passes through the vent. High injection port temperatures (300° C vs 250-280) produce a more consistent splitting of the higher boiling esters. This reduces the random errors at the expense of a slight loss in detection limit for the lower acids.

For six samples at 1 g/l concentration, the average % standard deviation for the seventeen calibrated acids was 1.97.

Figure 3 is a typical chromatogram of an oxidized solvent. C1 to C4 acids are not observed since they partition into the aqueous phase. The minor peaks between the linear esters are branched isomers. They were not individually identified or calibrated.
4) Pierce, Rockford, Illinois
MULTIELEMENT THERMAL IONIZATION ON DISCRETE PARTICLES FOR GEOLOGICAL DATING

Michael J. Kristo, The Mcllolan Central Laboratory, Mcllolan AFB, CA, 95652-6437

We have developed a technique for dating microscopic particles, using the electron microprobe (EMP) for elemental analysis and sequential thermal ionization mass spectrometry (TIMS) on a three sector (BBC) mass spectrometer for isotopic analysis. This EMP/TIMS technique has several advantages over standard methods. The standard analysis for geological dating uses isotopic dilution mass spectrometry (IDMS) for each element of interest, e.g., U, Th, and Pb. These analyses are typically performed on bulk samples, although some work has been done on dating distinct particles using microdissolution techniques (1-2). Isotopic analyses are performed almost exclusively on single sector mass spectrometers, although one group has used a double sector (BB) mass spectrometer to date ocean basalts (3).

This standard technique has several advantages. First, isotopic dilution is one of the most accurate and precise methods of quantitation. Second, one can correct for isotopic fractionation during the analysis by double spiking the sample (4-5). Third, only a single analytical technique is used. However, the current technique has certain disadvantages as well. First, it is tremendously labor intensive, requiring chemical dissolution, spiking, elemental separation and purification, followed by separate thermal ionization analyses for each element of interest. Second, the chemical preparation leads to significant procedural "blanks." Even using the microdissolution technique, blanks are in the picogram to tens of picograms range. These blanks eventually limit the smallest sample which can be analyzed accurately. Third, the single stage mass spectrometer provides poor sensitivity to lower abundance isotopes, e.g., Th$^{230}$. A 2-stage mass spectrometer improves on this situation somewhat, providing abundance sensitivities around $10^{6}$ as opposed to around $10^{4}$ for single sector instruments.

The EMP/TIMS technique improves on the conventional technique in several areas. First, analyses are much faster. Thus, EMP/TIMS can be used a valuable survey technique. In fact, the EMP can be used as a very fast prefilter to select only the most interesting particles for further analysis. Second, direct analysis of microscopic particles virtually eliminates chemical blanks. Combined with the high ionization efficiency of TIMS and the high transmission efficiency of the three stage design, this technique achieves high overall sensitivity. Third, use of a three-sector mass spectrometer improves abundance sensitivity to greater than $10^{6}$. Drawbacks to this method are possible inaccuracies in electron microprobe data due to "small particle effects," lack of correction for isotopic fractionation (since spiking is not possible), and the necessity of a refractory matrix when analyzing Pb using single filament TIMS.

We recently analyzed a thorite sample of unknown origin using EMP/TIMS. The sample provide four decay schemes useful for dating each particle:

- $^{238}U\rightarrow^{206}Pb$ (1)
- $^{235}U\rightarrow^{207}Pb$ (2)
- $^{232}Th\rightarrow^{208}Pb$ (3)
- $^{230}U\rightarrow^{226}Th\rightarrow^{208}Pb$ (4)

Of course, decay schemes 1 through 3 occur through unstable intermediate products whose half-lives are short compared to the radioactive parent. So, after about 100 years, one can simplify the calculations for scheme 3 and neglect the intermediate decays. After 100,000 years, one can make the same approximation for scheme 2 and, after 1 million years, for scheme 1. Decay scheme 4 is only useful for dating samples less than about 400,000 years old. After 400,000 years, the level of Th$^{230}$ increases to such an extent that its decay rate equals its rate of formation from the decay of U$^{235}$. Secular equilibrium is established and the level of Th$^{230}$ remains constant. The equations for decays 1 and 2 can also be combined to date samples based only on the Pb$^{207}$/Pb$^{208}$ ratio (since the natural U$^{235}$/U$^{238}$ ratio is constant throughout the earth's crust). Therefore, it is possible to date particles using only mass spectrometric data. In fact, this can be the most accurate method of dating the particle in the event of recent loss of lead (6). We obtained the following results on particles from 40 to 150 μm in size:

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Percent relative deviations calculated from EMP and TIMS data using propagation of error analysis are given within the parentheses. Dashes (-) indicate particles which had insufficient ion currents to measure one or more of the elements. The variations in the calculated ages are systematic and are caused by either lead diffusion or episodic loss of lead (7-12). Plotting \( \text{Pb}^{207}/\text{U}^{235} \) vs. \( \text{Pb}^{206}/\text{U}^{238} \) for each datum yields a straight line which intersects the concordia curve (7-8) at 2.04 (+/-0.01) billion years.

Although not useful for dating this sample (being much older than 400,000 years), the \( \text{Th}^{230} \) levels from 1.5 to 2 ppm were measured. Despite the extremely low levels, the measured \( \text{Th}^{230} \) levels compared favorably with the expected equilibrium concentrations. Samples which did have large errors suffered from interferences from polyatomic ions as measured at background masses. Geochemical or geophysical fractionation can also lead to differences in the measured and theoretical \( \text{Th}^{230} \) concentrations.

In conclusion, EMP/TIMS allows fast geochronological surveys on microscopic particles, allowing quick characterization of granular samples. EMP/TIMS virtually eliminates interferences from chemical blanks, allowing us to look at smaller particles than IDMS. The increased abundance sensitivity of the three sector mass spectrometers allows measurement of large isotopic ratio characteristic of U/Th systems.

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THE USE OF TIMS/IDMS TO CHARACTERIZE THE EXTRACTION OF 44CALCIUM-ENRICHED BIOLOGICAL FLUIDS USING ION EXCHANGE MEMBRANE FILTERS

Nancy E. Vieira1, David L. Hachey2, and Alfred L. Yergey1

1National Institute of Child Health and Human Development, NIH, Bethesda, MD. 20892; 2USDA/ARS Children's Nutrition Research Center, Houston, TX 77030

We have been involved in the study of calcium absorption and kinetics in a variety of populations including premature infants, pregnant and lactating women, and patients with disorders of calcium metabolism. Oxalate precipitation has been the method used for the extraction of the calcium from serum and urine (1). However, this precipitation procedure requires 2 days to complete and may coprecipitate other cations that could interfere with the thermal ionization process of the extracted calcium. This interference may cause irreproducible sample volatilization which results in the occasional interruption of automated data acquisition that we observe principally with urine samples. Our objective in this study was to evaluate the use of ion exchange membrane filters for the extraction of a more uniform chemical form of calcium from the biological samples so as to minimize the irreproducibilities of volatilization. Parallel ion exchange and precipitation experiments were designed to test for the occurrence of isotopic fractionation.

The ion exchange membrane filters evaluated were Bio-Rex® 25 mm Sample Preparation Discs (Bio-Rad Laboratories, Richmond, CA) with: a) AG® 50W-X8 Cation Exchange Resin and b) Chelex® Chelating Ion Exchange Resin. The matrices investigated were: water, serum, and urine. Reagents used in the sample preparation were: deionized water, J.T. Baker Ultrex II® grade acids and base, and HPLC grade methanol . Isotope ratios were determined using the Finnigan MAT THQ Thermoquad Mass Spectrometer with electron multiplier detection in the dual filament mode.

Membrane filters were loaded with 1, 1, and 3 ml of water, serum and urine, respectively. Calcium was eluted from cation and Chelex® filters with 6 M and 1 M HCL, respectively. Three ml of eluant extracted most of the Ca from both types of filters. The results for total % recovery of the calcium loaded onto the membrane filters and directly precipitated are shown in Table 1. They demonstrate that cation filter Ca extraction is more efficient for water and urine samples. Comparable results for either method was observed for serum samples.

Duplicate standard curves of the sample matrices of a water standard (0.1 mg/ml Ca), serum (0.091 mg/ml Ca) and urine (0.045 mg/ml Ca) were prepared by the addition of highly enriched (98.95%) 44Ca tracer to result in 44Ca excesses approximating 10, 20, and 30%. Following a 24 hour equilibration, the calcium was extracted from one-third volume of each sample by precipitation, cation and Chelex® ion exchange. The serum (but not the water or urine) filters were washed with 10 ml of deionized water prior to elution of the calcium. Calcium was extracted with 2 ml of eluant. The elutions were then heated and dried in an Ar stream and dissolved twice in HNO3 and dried. All precipitates (oxalate, cation, Chelex®) were dissolved in 3% HNO3 for TIMS.

Table 2 shows the regression line comparisons of direct precipitation versus cation and Chelex® ion exchange A%excesses. Each line represents the regression of the duplicate curves for the respective extraction procedure. The results show that isotopic fractionation does not occur as a result of ion exchange extraction of calcium in these experiments.

The results of the assessment of automated sample analysis are shown in Table 3. Interruption of automated data acquisition of oxalate precipitated calcium is a problem principally seen with urine samples. An improvement in automated filament analysis was observed for water and urine calcium samples extracted with ion exchange filters.

1071
We conclude: 1) ion exchange membrane filters can be used for calcium extraction of biological fluids for TIMS, 2) sample preparation time is 1 day, 3) urinary calcium should be extracted using cation exchange filters, 4) Chelex® filters are to be used for calcium extraction from water or acid digests, 5) serum calcium can be extracted by using any of the 3 methods.


Table 1: % recovery of calcium from water, serum and urine using ion exchange membrane filters and oxalate precipitation.

<table>
<thead>
<tr>
<th>extraction method</th>
<th>water</th>
<th>serum</th>
<th>urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg Ca</td>
<td>1.0</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>% recovery</td>
<td>85.5</td>
<td>77.6</td>
<td>65.9</td>
</tr>
<tr>
<td>Chelex®</td>
<td>1.0</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>mg Ca</td>
<td>63.4</td>
<td>97.8</td>
<td>19.6</td>
</tr>
<tr>
<td>% recovery</td>
<td>93.0</td>
<td>100.0</td>
<td>96.3</td>
</tr>
</tbody>
</table>

Table 2: Regression line comparisons of oxalate precipitation versus ion exchange A%excesses for water, serum, and urine.

<table>
<thead>
<tr>
<th>precipitation vs:</th>
<th>regression line</th>
<th>*Sy.x</th>
</tr>
</thead>
<tbody>
<tr>
<td>water:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cation</td>
<td>y = -0.367 + 1.016x</td>
<td>0.330</td>
</tr>
<tr>
<td>Chelex®</td>
<td>y = -0.118 + 1.000x</td>
<td>0.419</td>
</tr>
<tr>
<td>serum:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cation</td>
<td>y = 0.041 + 0.976x</td>
<td>0.427</td>
</tr>
<tr>
<td>Chelex®</td>
<td>y = 0.573 + 0.952x</td>
<td>0.240</td>
</tr>
<tr>
<td>urine:</td>
<td></td>
<td></td>
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<tr>
<td>cation</td>
<td>y = -0.628 + 1.017x</td>
<td>0.483</td>
</tr>
<tr>
<td>Chelex®</td>
<td>y = -0.813 + 0.990x</td>
<td>0.548</td>
</tr>
</tbody>
</table>

*Sy.x is standard error of the estimate

Table 3: Comparison of Ca extraction methodology and automated sample analysis.

<table>
<thead>
<tr>
<th>matrix:</th>
<th>extraction</th>
<th>total # filaments analyzed automatically</th>
</tr>
</thead>
<tbody>
<tr>
<td>water:</td>
<td>precipitation (n=12)</td>
<td>75%</td>
</tr>
<tr>
<td></td>
<td>cation (n=12)</td>
<td>92%</td>
</tr>
<tr>
<td></td>
<td>Chelex® (n=12)</td>
<td>100%</td>
</tr>
<tr>
<td>serum:</td>
<td>precipitation (n=18)</td>
<td>94%</td>
</tr>
<tr>
<td></td>
<td>cation (n=18)</td>
<td>83%</td>
</tr>
<tr>
<td></td>
<td>Chelex® (n=20)</td>
<td>90%</td>
</tr>
<tr>
<td>urine:</td>
<td>precipitation (n=12)</td>
<td>58%</td>
</tr>
<tr>
<td></td>
<td>cation (n=14)</td>
<td>79%</td>
</tr>
<tr>
<td></td>
<td>Chelex® (n=16)</td>
<td>62%</td>
</tr>
</tbody>
</table>
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ISOTOPE RATIO MEASUREMENTS BY "WHOLE MOLECULE" MS: COMPARISON OF A QUADRUPOLE AND A MAGNETIC INSTRUMENT.
Pratt,D., Hanzlik,R., Williams,T.D.,Department of Medicinal Chemistry University of Kansas, Lawrence, Kansas 66045

Introduction

Kinetic isotope effects (KIEs) in the enzymatic conversion of substrate to products are frequently used as probes of reaction mechanisms. The usual methodology used to determine a KIE is to detect the small change in isotopic content caused by enzymatic discrimination in the isotopic variants of the substrate at natural abundance levels, requiring the use of an isotope ratio mass spectrometer.

This study presents an alternative methodology using "whole" molecule mass spectrometry to determine KIEs in the enzymatic hydration of epoxides to diols. KIEs are measured by following the change in isotopic content of partially labeled (deuterium or $^{13}$C) substrates. Label to unlabel ratios are kept close to 1:1 and the change in isotopic content from substrate to product is measured by observing the isotope cluster associated with the molecular ion and/or a major fragment.

Experimental

Quadrupole -Nermag R10-10 with SPECTRAL data system and a Girdel GC modified with a J&W scientific split/splitless capillary injector.
Magnetic -VG ZAB-HS with 11/250 data system and a Hewlett Packard 5790 GC
Chromatography - 15 or 30m of bonded phase capillary column
Sample size: 50-100 ng per injection
All experiments on the ZAB were by Voltage scan SIR

Emissions (µA) 200 100
Electron energy (eV) 70 70
Multiplier gain 3.5x10^3 5.7x10^3
Amplifier (amps for 10V) 10^-7 10^-7
Ion peak width 0.5-0.6 FWHT 800 resolution (flat top)
* variables on cycle time adjusted to give 15-20 cycles during a GC peak
ion measurement time(ms) 18, 38, 74 80-100
ion switch time .036 5
cycle switch time 168 na
Bromonaphthalene is used as a test case of a 1:1 isotopic mixture to determine the precision and accuracy attainable using capillary GC SIM MS techniques on a quadrupole and a magnetic deflection instrument. The theoretical ratio for the $^{81}$Br/$^{89}$Br molecular ions is 97.9% (208/206). Eight trials of five GC SIM injections each were done over 8 months. On the magnetic instrument the average 208/206 ratio was 97.2% ± 1% relative standard deviation for the trials and the range of deviations for each set of five injections was 0.1-0.4%. On the quadrupole the average of the trials was 95.4±1.2% and the range of deviations for individual trials was 0.3-1.0 %.

Other Compounds Tested: The magnetic deflection and quadrupole instruments were compared using the samples of $^{13}$C or deuterium labeled mixtures at label to unlabel ratios of 1:1. The test mixtures included: D0/D5 methylbenzoate, $^{13}$C/Nature abundance or D0/D2 p-phenystyrene acetone and $^{13}$C or deuterium labeled p-nitrostyrene epoxide. In all cases the magnetic instrument provided precision 2-5 times better than the quadrupole. The range of precision on the magnetic instrument for the $^{13}$C labeled samples was 0.2-0.7%, on the quadrupole the range for the same samples was 0.5-1.5%. The measurements using deuterium labeled samples were consistently 1.5-3 times less precise than from $^{13}$C labeled compounds. For the deuterium labeled compounds, measurements from the magnetic instrument remained 2-3 times more precise than from the quadrupole.

The KIE in the enzymatic hydration of p-nitrostyrene epoxide (PNSO) to diol was determined as outlined in figure 1. Also, a mixture of 1:1 natural abundance and $^{13}$C PNSO was spiked with natural abundance PNSO to provide samples with the range of isotopic content

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expected from KIEs in this reaction. The precision of the 5 injections of each standard was in the range of 0.1-0.3%. The results show that 2-3% change in isotope content can be detected reliably.

**Experimental Plan:**

Starting PNSO → Time = 0 aliquot → PNS-Acetamide

Incubate with MEH

10% Conversion → quench 90% of the reaction volume, isolate the dial, convert to PNS-Acetamide

100% Conversion → isolate dial, convert to PNS-Acetamide

Analyze samples A, B and C to determine 13C-enrichment

**FIGURE 1:** Enzymatic Application: Microsomal Epoxide Hydrolase (MEH)

**Results** from Enzymatic incubations

A) Compare 0-time to 100% conversion samples (expect NO CHANGE in 13C-enrichment)

208/209 ratio (12C/13C), relative standard deviation (%)

<table>
<thead>
<tr>
<th></th>
<th>0-Time</th>
<th>100% conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>R(-)-PNSA</td>
<td>92.5±0.3</td>
<td>92.7±0.4</td>
</tr>
<tr>
<td>S(+)-PNSA</td>
<td>94.0±0.1</td>
<td>93.5±0.3</td>
</tr>
</tbody>
</table>

Conclusion: No difference in 13C observed (as expected)

B) Isotopic Discrimination During Enzymatic Hydration

<table>
<thead>
<tr>
<th></th>
<th>208/209 %conversion</th>
<th>208/209 %conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>R(-)-PNSA</td>
<td>96.6±0.2</td>
<td>96.8±0.1</td>
</tr>
<tr>
<td>S(+)-PNSA</td>
<td>9.2</td>
<td>9.0</td>
</tr>
</tbody>
</table>

The apparent Kinetic Isotope effects are:

<table>
<thead>
<tr>
<th></th>
<th>208/209 %conversion</th>
<th>208/209 %conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>R(-)-PNSA</td>
<td>96.8±0.1</td>
<td>2.3</td>
</tr>
<tr>
<td>S(+)-PNSA</td>
<td>98.3±0.2</td>
<td>97.8±0.5</td>
</tr>
</tbody>
</table>

The KIEs determined here agree within experimental error to the values determined for the same system using an isotope ratio instrument.

**Conclusions:**

With proper controls and experimental design, this method is adequate for measuring many small KIEs (e.g. secondary 2H or primary 13C, 18O, or 15N) which are of interest to mechanistic enzymologist.

**Acknowledgments:** The authors wish to acknowledge the technical assistance of Mr. Bob Drake and the valuable comments and advice of Dr. Charles M. Judson on quantitation by MS. Funding has been provided by NIH GM-21784 and GM-31910.
data, denoted by $o$, was fitted to the theoretical curves. In the case of simple background (Fig. 3a), the only background effects and combined reduction/background effects for pressinolc acid. The experimental contributor to the elevated ($A+2J/A$) values measured. Figure 3 presents the calculated curves considering the dependence of the extent of reduction with concentration would imply that reduction cannot be the sole cause the greatest increase in the measured ratios. It was assumed for these calculations that the analyte signal is little attention, however, is the interference of "peak-at-every-mass" signal due to beam-induced chemical damage. The aim of this work was to develop a method for quantifying that the beam-induced background contribution to the isotopic peaks so that it could be removed from experimental values, thereby providing a true representation of the extent of analyte reduction alone. Mass spectrometric measurements were made using a VG Autospec-Q spectrometer. The VG FAB/LSIMS source was equipped with a caesium ion gun. In a typical experiment, sample solutions were prepared in glycerol and 2 µL portions were then placed on the probe tip using a syringe. Isotopic peak ratio measurements were made using narrow voltage scanning and multi-channel acquisition over a period of 5 minutes in order to maximize reproducibility.

Bombarding beam density values were measured using a Faraday cup detector placed at the target.

Several parameters have been studied independently in attempts at gaining insight into the reduction processes believed to be responsible for the observed phenomenon. Surprisingly, the chemical background interference, which results from interactions of analyte and matrix molecules with the fragments or electrons produced during the bombardment event, was not given much consideration until recently. The parameters which could reflect changes in the signal/background ratio (sample concentration, beam energy and primary beam flux) were examined. A series of experiments were conducted at constant beam density (0.03 µA/mm$^2$) where concentration and beam energy were varied independently. Figure 1 illustrates the variation in $(A+2)/A$ values relative to the concentration of met-enkephalin (Fig. 1a) and pressinolc acid (Fig. 1b) at different primary beam energies. It can be seen from this figure that the bombardment energy has little, if any, effect on the ratios measured. This data is contrary to results reported by Kazakoff et al. which suggest that the concentration effect is eliminated by decreasing bombardment energy. It should also be noted in Figure 1 that the measured isotopic ratios are consistently higher at the lower concentrations, regardless of the bombardment energy. This trend could be attributed either to a poor S/N ratio or to an enhanced amount of reduction occurring in the more dilute solutions. It would be expected, however, that reduction of the analyte be favoured in the more concentrated solutions where the population of analyte subjected to the beam would be higher. The effect observed, therefore, appears to reflect the decrease in analyte signal with respect to chemical background, not a greater extent of reduction occurring at the lower concentrations.

The effect of primary ion flux on the $(A+2)/A$ values was also studied. Figure 2, which presents the variation of isotopic peak ratio values for a pressinolc acid sample (0.005M) measured at various beam densities and beam energy of 24 KeV, shows that a small increase in beam density has a substantial influence on the measured ratios. These results indicate that the beam density rather than energy is promoting reduction or increasing radiation damage. In order to determine the relative contributions of reduction processes and background interference to the peak enhancement effect, the system was simulated mathematically. The relative increases in reduction and background contributions to the $A+n$ peaks would occur experimentally by increasing beam densities. Three possible causes for the inflated isotopic peak ratios were considered: simple background increase, reduction processes in the analyte alone, and a combination of reduction/background interference. It was assumed for these calculations that the analyte signal is linearly related to the sample concentration in the range studied. The background interference, reduction, and combined background/reduction effects were then applied individually at each relative concentration. It is reasonable to expect under these conditions that: a) without any background interference or reduction effects, the isotopic ratios would not change with concentration, b) an increase in background signal will cause higher isotopic ratios as the beam density is increased, c) an increase in the extent of reduction for the analyte would appear at higher concentrations, and d) combined reduction and background effects would cause the greatest increase in the measured ratios.

The dependence of the extent of reduction with concentration would imply that reduction cannot be the sole contributor to the elevated $(A+2)/A$ values measured. Figure 3 presents the calculated curves considering only background effects and combined reduction/background effects for pressinolc acid. The experimental data, denoted by $o$, was fitted to the theoretical curves. In the case of simple background (Fig. 3a), the
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Experimental points could not be fitted properly while for combined reduction/background effects (Fig. 3b), a very good fit was achieved with the theoretical curve representing 2% background and 2% reduction (denoted by A in Fig. 3b). It is obvious from this example, that both reduction and background interference are playing a role in the distortion of measured (A+2)/A values. Once the background contribution is evaluated, its removal should provide (A+2)/A values containing only the natural isotopic contributions and the signal due to reduction of the analyte alone. The experimental data for pressinoic acid was background corrected by 2% as determined in Fig. 3b. The raw and corrected data are shown in Figure 4. The corrected data can be used to quantify the extent of reduction which can be expressed as the variation of corrected values from theoretical values. The isotopic ratio values at very low concentrations remain above the plateau region of the curves despite background removal. This effect is due to an increase in the error of measurement where the analyte signal is very low. For this sample, the extent of reduction was found to be in the order of 2%. The background removal method described, therefore, should allow an accurate representation of the quantity of reduction occurring in different combinations of analyte and matrix.

Effect of Sample Preparation Temperature on D/H Isotope Ratios in Water Samples

L. J. Joseph, G.G. Dolnikowski
USDA-HNRCA at Tufts University, Boston, MA 02111

Introduction
Quantitative reduction of D$_2$O/H$_2$O mixtures to H$_2$ and HD by zinc at high temperature is a popular sample preparation technique prior to mass spectrometric measurement of the D/H isotope ratio in geological and biological samples. A number of different temperatures and types of zinc are currently being employed by mass spectrometry labs around the world. Therefore, we have prepared samples using four different lots of zinc over a range of temperatures from 440°C to 500°C to determine the optimum conditions for the zinc reduction method. All of the samples we prepared were run versus an NBS standard, Standard Mean Ocean Water (SMOW). We used 4 samples, a deuterium-depleted NBS standard, Greenland Ice Sheet Precipitation (GISP), a baseline urine sample which has approximately the sample D/H ratio as SMOW, a deuterium-enriched standard prepared locally, and a deuterium-enriched urine sample collected from an adult who had been given doubly-labelled water to drink.

Experimental
A pyrex tube with a teflon stopcock (Ace Glass, Inc.) is filled with 120 mg of zinc (or 300 mg when using urine) in the tube is evacuated. The tube is flushed with dry nitrogen while five microliters of water or urine is added to the tube. The sample is frozen with liquid nitrogen and the tube is re-evacuated. The stopcock is closed to preserve the vacuum above the zinc and the frozen sample. The tube is placed in a heating block and heated to the desired temperature (440-500°C). To ensure that all samples in a set were heated equally (to within 1°C), the temperature of each hole in the heating block was measured with a mercury thermometer and a digital thermocouple which had been calibrated to freezing and boiling water. The samples are left in the heating block until a grayish ring appears in the tubes just above the level of the heating block (0.5-1.0 hours).

Results
The deuterium enrichment of the samples is reported in delta units and calculated according to the formula:

$$\delta = 1000 \times \left( \frac{D_{\text{sample}}}{D_{\text{SMOW}}} - 1 \right)$$

The standard deviation of replicate analyses of a single sample on a VG SIRA-10 gas isotope ratio mass spectrometer employed for this study was in all cases less than 0.2 delta units, with D-enriched samples standard deviations in the 0.15 delta range and D-depleted samples standard deviations in the 0.07 delta range. These figures represent the internal precision of the instrument and are independent of the sample preparation method.

Figure 1 shows the behavior of GISP when it is subjected to sample preparation using different types of zinc over a range of temperatures. Figure 1A shows the temperature versus delta value curves of two lots of Analar BDH Zinc, and Figure 1B shows the temperature versus delta value curves of two lots of zinc manufactured in John Hayes' lab at the Indiana University. Figure 1 indicates that not only are the temperature versus delta value curves different for different types of zinc, but that there are significant differences between batches of the same kind of zinc.

Figure 2 shows the behavior of a deuterium-enriched standard using different types of zinc over a range of temperatures. Figure 2A shows the temperature versus delta value curve of Analar BDH zinc and Figure 2B shows the curve of John Hayes' zinc. Figure 2 indicates that the D-enriched samples have higher standard deviations than the D-depleted samples in Figure 1. Figure 2 also shows that whereas the standard deviation of the Analar BDH zinc increases with increasing temperature, the standard deviation of the John Hayes' zinc decreases with increasing temperature. We observed similar trends with baseline urine samples and D-enriched urine samples. Figures 1 and 2 also show that the accuracy of the analysis to the certified value of the standard is also strongly dependent on the temperature of sample preparation and the type of zinc chosen.

Conclusion
The delta values and the standard deviations of both enriched and depleted deuterium samples varied with the type of zinc, the lot number of zinc, and the sample preparation temperature. Therefore, it seems advisable to determine the optimum temperature for each new batch of zinc with a standard that has a D-enrichment similar to that of one's samples. The standard deviations we obtained for D-enriched samples were 5-35 times greater than the internal precision of the isotope ratio instrument. It would seem that there is considerable room for improvement in the precision of the zinc reduction technique for D-enriched water samples.
ISOTOPIC ANALYSIS BY TOF SIMS


Isotopic analyses require high precision and extreme dynamic ranges. Ion statistics alone dictates that $10^6$ ions must be detected for a possibility of 0.1% RSD on measurement of a single ion signal. Part per thousand or lower ratios therefore dictate that minor isotope signals be observed at levels that produce major isotope ion counts higher than $10^8$.

Traditionally these measurements have been made using Thermal Ionization, Glow Discharge or other types of mass spectrometry that utilize bulk vaporization of the metal sample during ionization. These instruments produce large ion currents which are measured with Faraday cup detection systems.

The vaporization of metal atoms from the bulk sample produces solid and vapor phase isotopic compositions that change with time as the sample fractionates. High precision and accuracy are achieved only by carefully reproducing sample loadings, temperatures and analysis times so that a Fractionation Bias correction can be applied to the data.

Another problem associated with traditional isotopic analysis of metals is carry-over. Evaporated material from one sample can condense nearby and be heated or sputtered back into the vapor phase to interfere with results from later samples. The traditional answer to the cross contamination problem is to make much of the ion source removable as part of the sample introduction assembly. These precision components must then be cleaned external to the ion source before use in running other samples.

There is now a need to run environmental samples where the loading is not known in advance, making fractionation corrections less accurate. There is also a growing interest in accurate isotopic analysis of light metals where carry-over, fractionation and any other isotope effects are the most troublesome.

**PROPOSAL**

The new low primary-ion-dose Time-of-Flight Secondary-Ion Mass-Spectrometry Instrumentation should be ideal for isotopic analysis. TOF SIMS need not involve bulk heating of the sample. Use of beam raster, short pulses and low currents can allow stable work at incident ion levels near and below the static SIMS limit ($<1\%$ or $<10^5$ surface site collisions per cm$^2$ at a rate just fast enough to expel adsorbed gasses).

SIMS ion collection optics allow nearly a square millimeter of sample surface to be in focus for mass analysis. Concentration of all the incident current into $<10^7$ second pulses, as required for TOF mass analysis, allows time for heat to dissipate between shots. The lack of bulk heating or sample vaporization along with direct ionization from the solid sample surface alleviate the two most bothersome problems traditionally associated with isotopic analysis. Fractionation is prevented since there is effectively no removal of material from the sample. And, since so little material is removed, effectively none is sublimed onto vacuum chamber components or other samples, eliminating carry-over.

Time of Flight mass analysis also has advantages necessary for measuring high precision isotope ratios. The ions in the ion packet extracted from each shot are mass analyzed according to differences in drift time and can all be quantified at the ion detector for each shot. This parallel detection scheme makes isotopic analysis independent of signal stability with all of the advantages of multiple Faraday detectors.

TOF is a more versatile detection scheme for isotopic analysis. TOF instrumentation collects thousands of channels over a wide mass range allowing simultaneous measurement of several isotope ratios and/or de-convolution of complex interferences. Traditional multiple Faraday magnetic instruments, on the other-hand, typically measure from two to ten channels over a range of only a few mass units.

Modern reflectron equipped TOF SIMS instruments easily achieve the 300 to 600 mass
resolution of traditional instruments and could even be set to much higher resolutions (>10,000) if required to solve problems with isobaric interference.

The modern TOF SIMS instrument can achieve the quantitative dynamic range required for isotopic analysis. The ion counting electronics on the instrument used here saturate at 8 counts per channel per shot. The primary ion gun and mass analyzer can be cycled at one million shots per minute giving about a $10^5$:1 dynamic range for a single channel per minute of integration time. The experiment can be set up so that each mass signal of interest is spread over 10 or more channels to give $10^6$:1 dynamic range per mass of interest per minute. Therefore, integration times well under an hour should be sufficient for the $10^7$ dynamic range needed to solve the hardest isotope quantitation problems.

EXPERIMENTAL

We used a KRATOS / Cambridge Mass Spectrometry Limited PRISM TOF SIMS instrument which was temporarily located at the KRATOS Analytical Applications Lab in Ramsey, New Jersey. This instrument was used with an ion extraction energy of 3KV and a drift energy of 4.5KV. The effective ion flight length is 3 meters including a two-stage one-meter Mamykin-type reflectron for energy refocusing. The ion detector is a two stage channel plate. LeCroy high speed multichannel analyzer electronics are used to count and time the ion events at the detector. The user interface for experiment control and data presentation is an HP 9000 series computer utilizing 32 bit Workstation Basic.

SET-UP: The primary ion beam was 25KV Ga⁺ produced from a liquid metal ion gun. The effective spot size during these experiments was about 1 micron and could be rastered to cover up to a one square millimeter area within the focus of the ion optics. The gun was operated at 600 pico-amps with a gate-on pulse width set up to 100 nano-sec.

The experiment was run at a 10KHz repetition rate so that the ion current was 0.6 pico-amps at the sample surface. Alternately the primary ion current can be expressed as $6 \times 10^{-17}$ coulombs or about 375 ions per shot. This primary ion current level was selected because it was sufficient to give between 1 and 10 detected ions per shot.

Sample Presentation: Our samples were all deposited as spots dried from solution onto a 3.5cm Dia. aluminum foil disk. The samples were non-charging, allowing use of a static extraction field with no need to utilize the instrument’s versatile charge neutralization capabilities.

Two lithium solutions were used for these tests. One solution was highly basic and contained lithium that was highly enriched in $^6\text{Li}$ (about 93 atom %). The other solution had a more neutral PH and contained Li enriched to about 95 atom % $^6\text{Li}$. One of the $^6\text{Li}$ spots was placed near the $^7\text{Li}$ spots to check for cross contamination as is shown on the photograph.

CONCLUSIONS & OBSERVATIONS:

The precision obtained for the one million shot lithium isotope analysis was near the theoretical predictions based on the number of ions detected. This indicates that essentially any required precision and dynamic range might be obtained, granted sufficient time for integration of the signal.

The data taken at higher current densities seems to indicate that some form of fractionation with preferential enhancement of the $^7\text{Li}$ signal is possible with a Ga⁺ beam. We expect to minimize these effects with an O⁺ beam.

Analysis of the foil background shows that the near neutral solution spread over the entire foil apparently by capillary action along surface cracks. The strongly basic solution however reacted strongly with the Al foil apparently stabilizing the water via the hydration of aluminum oxides.

The deviation of the isotopic analysis of Li on uncontaminated foil from the accepted natural abundance (97.3% $^7\text{Li}$ measured as opposed to 92.58% reported) indicates that more work needs to be done to seek out sources of bias.

The variation of the isotopic analysis with changing raster area and primary ion current may indicate the expected requirement for low energy to prevent fractionation. However, the next experiment must be to use an oxygen beam from a Penning Ion Gun to get the same ion yield at much lower currents and with much lower total energy transfer to the surface.

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DIRECT MASS DETERMINATIONS OF NEUTRON DEFICIENT NUCLEI CLOSE TO $^{100}$Sn

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1. INTRODUCTION

The mass of a nuclide is one of its most fundamental properties. A systematic knowledge of atomic masses provides an abundance of nuclear-structure information. Various techniques have been used to measure atomic masses. One of the most reliable and precise methods has been direct mass measurements made with high-resolution, double-focusing mass spectrometers. Unfortunately, this technique is only readily applicable to naturally occurring nuclides. For nuclei far from stability, mass information has, in the past, been obtained predominantly with decay-energy measurement techniques, which require detailed knowledge of the decay schemes of the nuclides investigated. However, recent technical advances have opened up the possibility of routine direct mass-spectrometric determination of the masses of such exotic atoms. We describe here the results obtained with one such method, based on the high-resolution Chalk River on-line isotope separator.

Considerable interest has been shown recently in the masses of nuclides in the vicinity of the proposed doubly magic $^{100}$Sn. Information on nuclear masses in this region makes it possible to test the theories of nuclear masses and to delineate nuclear structure near a double shell closure. In one case of particular interest, Wouters et al. have reported large discrepancies between their results for $^{105}$In and values arrived at from mass formulae and systematics for nuclei near $^{100}$Sn. Subsequently, the $\beta$ spectra from all three isotopes were re-measured. The results of these recent measurements combined with level-scheme information yield $Q$-values for $^{105}$In and $^{106}$In and, in a less direct manner, one for $^{106}$In as well.

We have directly measured the atomic-mass ratios for $^{103,104,105}$In, $^{106,107,108}$Sn and $^{102,103}$Cd using mass-spectrometric techniques, with the Chalk River on-line isotope separator. Our results are in good agreement with both the results of systematics and mass formulae, and are consistent with the recent results of Bom et al. A preliminary report on our work has been presented earlier. The values presented here are consistent with but supersede those given in that report.

2. EXPERIMENTAL PROCEDURE

A detailed description of our mass-measurement apparatus has already been published. The radioactivities of interest were produced by the bombardment of a target, placed near the entrance window of a FEBIAD ion source, with a heavy-ion beam from the TASCC accelerator. The reaction products recoil out of the target, through a few heat shields and are implanted into a low-density graphite catcher inside the ion source. They subsequently diffuse out of the catcher and are ionized in the plasma region of the source. An ion beam is extracted and formed by the extraction potential (nominally 40 kV) applied to the source. The ion beam is then analyzed by the separator magnet and brought to a focus at an image slit. With an ion-source exit orifice of 0.5 mm and an image-slit width of 0.25 mm, a resolving power ($M/\delta m$, where $\delta m$ is the FWHM of the mass peak corresponding to the mass $M$) of 7,000 was achieved for the measurements described here. Ions that pass through this slit are guided through an electrostatic beam-transport line into a low-background area where they are implanted into an aluminized mylar tape of a tape-transport station. At the end of a preset interval, the activity collected on the tape is moved in front of a HPGe detector where the gamma-ray spectrum of the sample is recorded.

To measure the mass ratio between two species, the ion beams corresponding to the selected masses are swept across the image slit by changes of the extraction potential in a series of pre-programmed steps. At each of these steps a gamma-ray spectrum is taken for the ions transmitted to the tape station and the corresponding extraction voltages are recorded. In this manner, each of up to three mass peaks may be scanned over. The duration of each step is dictated by the half lives of the activities involved.

The raw data consist of numerous gamma-ray spectra and their corresponding values of the extraction potential. We generate a mass spectrum by plotting the area of selected gamma-ray peaks against the applied voltages. Because of the gamma-ray tagging, it is possible to generate mass spectra that are free of contamination from unwanted isobars or isomers even when the resolving power of the separator is insufficient to resolve the untagged mass-spectral peaks.

The centroid voltages of the generated mass-spectral peaks are used to calculate the mass ratio between two species through Bleakney's theorem, which states that two ions of masses $M_i$ and $M_j$ will follow identical trajectories through the separator if all magnetic fields are kept constant and all electric potentials applied to the instrument are changed so that:

$$M_i V_i = M_j V_j \quad (1)$$
Therefore, from a knowledge of the centroidal voltages, $V_1$ and $V_2$, and one of the masses, it is possible to calculate the unknown mass.

Until recently, the mass scans were conducted in a sequential manner; i.e., the data points were accumulated in order of increasing (or decreasing) voltage. Thus, first one mass and then another would be scanned across the slit and detected. A slow drift in any one of several crucial components of our system would therefore have had a sizable impact because of the relatively long time spent completing the scan over one mass before data collection began on the next. In order to average out the effect of such drifts, this sequential method has been replaced by interleaved scans. Now data collection alternates between points corresponding to each of the two (or three) different masses being scanned. In addition to interleaving the scans over the various peaks, the sequence also alternates between either side of each individual peak. All peaks are therefore observed quasi-simultaneously, greatly reducing the likelihood of systematic errors arising from slow drifts.

3. CONCLUSION

The mass ratios determined by us have been combined with the auxiliary data to yield values for the previously unknown atomic masses of $^{107}$Sn and $^{102}$Cd as well as the less well known atomic masses of $^{104}$In. The results are given in Table 1. These values are in excellent agreement with values that are based only on systematics. The results for $^{107}$Sn and $^{102}$Cd are preliminary. Our result for $^{104}$In may also be combined with the known mass of $^{106}$Cd to yield a Q value for $^{104}$In of 7938 (140) keV. This value disagrees substantially with an earlier result by Wouters et al. of 7260 (250) keV but is seen to be in good agreement with systematics (as demonstrated by the agreement of the deduced mass) and the value of 7800 (250) keV recently determined, via an indirect route, by Bom et al.

An examination of $S_{2n}$ values provide a means of studying nuclear binding energies for nuclei in this region. The results of Wouters et al. show unexpected features at N = 58. When these results were published they represented the nuclides with known mass closest to the doubly magic nuclide, $^{100}$Sn, and their odd behavior stimulated further research into the atomic masses in this region. With the inclusion of the new values from this work and the work of Bom et al., no evidence of any unusual behavior in the systematics of atomic masses for these nuclides is observed.

The results described here represent the first such to be accomplished after the move of our separator to its new location in the TASCC facility. Recent improvements of the separator stability and changes in our mass-measurement procedures have led to an increased precision in our directly determined masses. We have directly determined the mass of $^{104}$In to an accuracy of 1.2 ppm and find that our value agrees with and substantially improves upon the recent result from Bom et al.

4. REFERENCES

MASS SPECTRAL CHARACTERIZATION OF FOULANT DEPOSITS FORMED DURING COMMERCIAL PHTHALIC ANHYDRIDE PRODUCTION

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Solid, foulant deposits taken from different points in a commercial phthalic anhydride plant were analyzed by Thermal Extraction-GC-MS and by Thermal Degradation-GC-MS. Samples were thermally extracted by heating at temperatures up to 330°C in an all fused quartz ThermEx instrument manufactured by Ruska Laboratories. Thermal degradation of samples was accomplished by heating up to 750°C in the same instrument. Compounds evolved from the deposits during heating passed through a heated transfer line (300°C) where they were cryofocused on the head of a 30m x 0.32mm DB-5 capillary GC column at -45°C in the oven of a Hewlett-Packard 5890 Series II GC. The detection of compounds eluting from the gas chromatograph was accomplished with either a Hewlett Packard 5970B or a 5971A MSD scanning over a 34 to 650 amu mass range once per second during GC column temperature programming.

Three types of foulant deposits have been identified. Type I is composed of a complex mixture (Figure 1, for example) of condensed aromatic ketones (e.g. 9-fluorenone, 9,10-anthracenedione), aromatic acid anhydrides (e.g. trimellitic acid anhydride), and isobenzofuran-based compounds. Mass spectra of these compounds typically contain fragment ions representing multiple losses of carbon monoxide and/or carbon dioxide (Figures 2,3). Loss of anhydride groups are easily recognized by a difference of 72 amu between major fragment ions. Type II deposit consists of various salts including sodium benzoate, sodium phthalate, and disodium phthalate. Thermal decomposition of such salts leads to the formation of a characteristic suite of compounds such as the parent acid of the sodium salt and a series of polynuclear aromatic hydrocarbons such as biphenyl, terphenyl, quaterphenyl, and various aromatic substituted fluorenes. The exact nature of Type III deposits is not known but they may consist of a mixture of phthalic anhydride and iron phthalates. Thermal decomposition of such deposits produces aromatic ketones, phenyl substituted aromatic hydrocarbons, and isobenzofuran compounds.

FIGURE 1
TOTAL ION CURRENT CHROMATOGRAM OF A TYPE I DEPOSIT
FIGURE 2
MASS SPECTRUM OF BIPHThALYL
(Note Multiple Losses of Mass 28, Carbon Monoxide)

FIGURE 3
MASS SPECTRUM OF A DIANHYDRIDE COMPOUND
(Note Multiple Losses of Masses 28 and 44)
CHARACTERIZATION OF IEC AND SEC CHROMATOGRAPHIC FRACTIONS FROM ASPHALTS USING THERMAL CHROMATOGRAPHY COMBINED WITH MASS SPECTROMETRY

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Using thermal chromatography coupled with flame ionization detection (TC/FID) and with gas chromatography and mass spectrometry (TC/MS), we have studied the chemical composition of a number of fractions of paving asphalts obtained by ion exchange chromatography (IEC) and by size exclusion chromatography (SEC). The objective of this work is to develop thermal chromatographic methods for determining gross differences in chemical composition and structure of asphalt cements. Chemical components include aromatic and aliphatic entities and polar functional groups. "Structure" in this context refers to the nature of the intermolecular assemblies which form in the neat asphalt.

Core asphalts from the Materials Reference Library of the Strategic Highway Research Program (SHRP) were obtained and fractionated by IEC and SEC at the Western Research Institute. Samples were subjected to computer-controlled heating in the Ruska ThermaChrom instrument. Species evolved during the heating process were delivered in a stream of helium to the FID or to the GC/MS (Hewlett-Packard 5985B) for analysis.

The thermal profiles obtained from TC/FID characterization of asphalt fractions show differences in the amounts of material being evolved and the temperatures at which the evolution occurs. Fig. 1 shows thermal profiles of IEC neutral fractions from three diverse asphalts. AAM-1 underwent a solvent de-asphalting process at the refinery: TC/FID and TC/MS data of the neutral fraction indicate this process removes semivolatile, low molecular weight asphaltenes. For a given asphalt, differences are observed in the composition of the IEC neutral, strong acid, and strong base fractions, shown in Fig. 2.

TC/MS data have been obtained from IEC and SEC fractions using a one slice method. Gross differences in composition among the fractions are readily apparent. For example, Fig. 3 shows TC/MS data from the strong acid fraction of asphalts AAA-1 and AAG-1. The strong acid fraction of AAA-1 is

Figure 1. TC/FID analyses of IEC neutral fractions from three asphalts.
observed to have a greater thiophenic content than the strong acid fraction of AAG-1. Interestingly, this finding is consistent with the levels of sulfur in the two fractions, determined by elemental analysis.
CHARACTERIZATION OF LOW-VOLATILITY HYDROCARBONS IN SOILS BY PROBE MICRODISTILLATION/MASS SPECTROMETRY

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INTRODUCTION

Petroleum, in the form of crude oil and refined products, is responsible for the most common organic contaminants found in soil. By far, the largest number of known pollution sites have been traced to leaking underground tanks used for storing gasoline and diesel fuel (1).

Over a period of time, petroleum contaminants in soil undergo significant changes in composition. These are a result of "weathering" caused by evaporation, dissolution, and biodegradation. Although accepted methods of analysis may be adequate for identifying compounds from recent contamination, as the pollutants age in the soil they become less recognizable. Therefore, to comply with anticipated future environmental regulations, new methods of analysis are required that are capable of identifying the larger molecules of lower volatility left behind as the lighter contaminants are either leached away, dissipated by evaporation, or converted to more stable products by chemical reaction.

Probe microdistillation/mass spectrometry is well suited for producing analytical data on low-volatility mixtures, such as those resulting from the weathering of petroleum-contaminated soil (2,3). The method could be applied just as well to the characterization of petroleum contaminants in marine and fresh-water sediments or of tar balls found along beaches as a consequence of oil spills at sea.

The capability of PMU/MS to distinguish contaminated from non-contaminated soil is illustrated with results obtained from the analyses of two soil samples. The first was taken from a site known to be contaminated with crude oil and the second (control) from a non-contaminated residential site. Mass spectra of the two samples were dramatically different, showing that more than enough information was provided to identify hydrocarbons in the contaminated soil and to distinguish these compounds from naturally occurring organic compounds, such as humic acids, in the non-contaminated soil.

EXPERIMENTAL

All data were acquired with a Kratos MS-50 high resolution mass spectrometer. The soil from the petroleum-contaminated site was introduced into the mass spectrometer via a cooled/heated quartz probe over a temperature range from -60 °C to +201 °C with programming by ion-current feedback. No preliminary sample preparation was performed. A sample weighing approximately 70 mg was loaded. Ions were generated by 70 eV electron impact and separated at a dynamic resolving power of 10,000.

The soil from the residential location contained a large amount of water, consequently, it had to be dried before introduction into the mass spectrometer. The water was removed by storing the sample overnight in a desiccator containing calcium sulfate. In addition, the sample was pulverized to maximize surface area. No other preparation was undertaken prior to analysis. The same method of introduction and instrumental conditions were used as for the petroleum-contaminated soil, except that temperature was programmed linearly to distill the sample over a range from -80 °C to +600 °C.

RESULTS AND DISCUSSION

Very strong peaks were evident for aliphatic hydrocarbons (m/z 71, 85, 99, ...) and alicyclic hydrocarbons (m/z 83, 97, 111, ...) in the mass spectrum of the contaminated soil recorded at a probe temperature of 198 °C. In addition, significant peaks for alkylbenzenes were present in the spectrum at m/z 77, 91, 105, ..., as well as peaks for other aromatics. Taken together, these peaks were more than adequate to identify petroleum in the soil sample. In contrast, the spectrum of the non-contaminated soil (control) recorded at a probe temperature of 185 °C was entirely different. Although a few peaks in the spectrum originated from hydrocarbons (e.g., m/z 91), other peaks corresponded to compounds containing oxygen. The latter types were represented, for example, by peaks at m/z 82 (C₂H₄O₂), m/z 96 (C₃H₆O₂), and m/z 98 (C₄H₄O₂). Elemental compositions were confirmed by high resolution mass measurements. Oxygen-containing compounds probably arose from humic material in the soil (4).
Peaks observed at m/z 207 and m/z 281 in the control had elemental compositions of C_{6}H_{15}O_{3}Si_{3} and C_{7}H_{21}O_{9}Si_{4}, respectively. They identified silicones in the sample. The only plausible explanation for silicones in the soil is that they originated from high-vacuum silicone grease used on the glass seal of the desiccator in which the control sample was dried. That is, silicones were adsorbed onto the soil by gaseous transfer from the grease. Although the limit of detectability for silicones was not established, this observation provides evidence of the very high sensitivity of PMD/MS.

A recent modification to the software used to process PMD/MS data permits calculation of apparent Arrhenius activation energies and frequency factors for desorption processes (3). First order kinetics is assumed (5). The calculated values are useful in defining the binding energy which must be overcome when compounds are desorbed from the surface into a vacuum.

A plot of ion intensity vs. temperature for m/z 91, C_{7}H_{7}, from spectra recorded for the non-contaminated soil was made. At least two minima were seen, corresponding to independent desorption processes. The first of these occurred between 450 K and 500 K and the second between 700 K and 900 K. Although the lower-temperature portion of the curve was not defined well enough to allow calculation of the kinetic quantities, its position on the temperature scale indicated that a process was occurring in which one or more aromatic compounds were being desorbed from the soil by rupture of relatively weak bonds.

Between 700 K and 900 K, the curve was smooth enough to allow calculation of an apparent activation energy and frequency factor from the equations for desorption. Values of 33.0 ± 7.2 Kcal/mol and 1.20 x 10^{6} s^{-1} were obtained. The magnitudes of these values are not explained by simple desorption as described by first-order kinetics. More likely, they describe a complex model in which one or more aromatic compounds were being desorbed by a process involving rupture of covalent bonds and diffusion through an organic matrix adsorbed onto the surface of the soil.

CONCLUSIONS

Probe microdistillation/mass spectrometry provides a very sensitive method for detecting low-volatility organic compounds in soil. When used with a high resolution mass spectrometer, the method readily distinguishes between hydrocarbons in the soil and naturally occurring organic compounds, such as humic acids. No sample preparation is required other than desiccation and pulverization.

Ion intensity vs. temperature data acquired in PMD/MS experiments can be analyzed to provide apparent Arrhenius activation energies and frequency factors for the desorption of compounds into a vacuum. These quantities are useful for defining a model by which compounds are bound to the surface of the soil and the pathway through which they are released.

LITERATURE CITED


Quantitative Determination of Triterpanes, Steranes, and Aromatized Steranes in Petroleum.

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Introduction: Organic compounds known as biological markers have played an increasingly important role in petroleum chemistry during the last 10 to 15 years. Biological markers are organic compounds detected in the geosphere whose basic skeleton suggests a link with a known natural product. Biomarkers are used to help characterize the source, thermal history, depositional environment, migration history, and extent of biodegradation of petroleum.

The majority of gas chromatography/mass spectrometry methods of biomarker analysis ignore absolute concentrations and rely on ratios to assess various maturation parameters. A technique has been developed to quantify selected petroleum biological markers. The method entails fractionating oils into aliphatic and aromatic components using an HPLC technique. Biomarkers from both fractions are quantified based on surrogates, internal standards, and external calibration standards by GC/MS in selected ion monitoring.

By quantifying biomarkers, crude oils may be correlated by visual methods and ratios based on peak heights or areas, and also on absolute concentrations of biomarkers. Advantages of quantitating biomarkers include: 1) direct intercomparisions between instruments; 2) maturity ratios using different ions will not be affected by mass discriminations; and 3) absolute amounts of biomarkers for downhole trends and basin evaluations.

Methods: A known amount of oil, approximately 50 mg and 500 ml of the surrogate standard were carefully added to the top of a ten centimeter by two centimeter column packed with alumina (80/200 mesh). Oil was eluted from the alumina column with 15 ml of dichloromethane and collected in 20 ml vials. The elutriate was then gently evaporated to 0.5 ml with purified nitrogen. The prepared samples were fractionated using an HPLC. This method separates the prepared samples into aliphatic and aromatic components using two Whatman Partisil 5µm PAC analytical columns and a solvent gradient from 100% hexane to 100% dichloromethane. The fractions were evaporated to 0.1 ml with purified nitrogen. Aliphatic fractions were subjected to adduction with 5Å molecular sieve beads to remove the n-alkanes prior to injection.

Prior to analysis of the aliphatic and aromatic fractions, the GC/MS was calibrated with the appropriate multi-component calibration standard to obtain response factors. The aromatic fraction was separated on a 30m x 0.32 mm i.d. DB-17 capillary column, and the aliphatic fraction was separated on a 30m x 0.32 mm i.d. DB-5 capillary column.

Results: One hundred and eleven oils and condensates from two oil fields offshore of Louisiana (South Marsh Island - 128 and Eugene Island - 330) and one onshore oil field in south Texas (Sarita Oil and Gas Unit) were analyzed for their biomarker composition. Total biological markers refer to the sum of steranes, triterpanes, and aromatized steranes with 20 to 35 carbons. The biomarker compounds in the Sarita oils contained 70.1% triterpanes and 19.4% steranes. 0.4% mono-aromatic steranes, and 4.6% tri-aromatic steranes. Total biomarker concentrations ranged from 49 to 2800 ppm with an average of 1138 ppm. The biomarker compounds in the Eugene Island-330 oils were comprised of 26.1% triterpanes, 59.8% steranes, 4.2% mono-aromatic steranes, and 7.3% tri-aromatic steranes. Total biomarker concentrations ranged from non-detectable to 2100 ppm with an average of 1475 ppm. South Marsh Island-128 oils exhibit the lowest concentrations of biomarkers of the three fields studied, ranging from 130 to 426 ppm with an average of 244 ppm. Total biomarker concentrations were 11.7% triterpanes, 79.5% steranes, 0% mono-aromatic steranes, and 4.0% tri-aromatic steranes.

Conclusions: The quantitative abundance of biomarkers can be used to distinguish these three fields. Quantitative differences in concentrations of biological markers in similar oils can be
due to variations in organic matter inputs to source rocks, processes that remove or alter selective fractions of petroleum, and thermal cracking of compounds during maturation. Quantitative determinations are potentially useful as conservative markers to detail losses during alteration, identifiers of precursor/product relationships in chemical reactions, and precise and accurate measurements for three-dimensional, mass concentration basin models. The relationships between biomarker concentration and bulk organic carbon must be defined in order to provide quantitative estimates of organic matter inputs to describe depositional and/or oceanographic settings.

Figure 1. Relative abundances of total biomarkers, terpanes, and steranes from SMI-128, EI-330, and Sarita Field.
A DYNAMIC BATCH INLET FOR GROUP TYPE ANALYSIS BY MASS SPECTROMETRY
By E. J. Gallegos and E. C. Pazzi

ABSTRACT
A new miniature dynamic batch inlet has been designed for group-type analysis of petroleum hydrocarbons. This system is designed to replace all other large, all-glass, glass-valved, free-standing batch inlets used previously. The new system uses an expansion bulb with an inside volume of only 2 cc. This bulb is placed in the oven of a gas chromatograph set at 300°C with one end connected to the injection port of the GC and with the other coupled directly to the source of the mass spectrometer. Hydrogen is used as the carrier gas at a flow of about 1 cc/min. Injection volume of the sample is between 0.02 and 0.03 µL using a 1-µL syringe. Acquisition time is just over a minute, with a total turnaround time including calculations of about 5 to 10 minutes.

EXPERIMENTAL
This work was done using a Hewlett-Packard 5790A gas chromatograph coupled to our VG 7070E or 70SE double focusing mass spectrometer. A small volume expansion bulb is coupled to the injection port of the GC via a 0.3-meter long, 0.25-mm ID uncoated fused silica column, whereas the other end was threaded to the ion source of the mass spectrometer via a meter long 0.1-mm ID fused silica capillary column. A schematic diagram of this system is shown in Figure 1. Hydrogen, set at a head pressure of 5 psi, is used as the carrier gas. The oven, injection port, and transfer line were all set at 300°C. The injection port is set to the splitless mode. Typical scan cycle time, 5 sec.

RESULTS AND DISCUSSION
Figure 2 shows the elution trace and mass spectra generated by using injection volumes of the 22 x 22 standard of 0.05, 0.1, 0.2, 0.4, and 0.8 µL. Using the 2 cc bulb, the sample remains in the source of the MS for about 1 min. for a 0.05-µL injection. There is a slight boiling point bias in these elution curves, but by summing across the top of the elution curve, the bias is completely eliminated.

Figure 3 shows the field ionization mass spectrum, obtained using the dynamic batch, of an atmospheric equivalent 450°C to 550°C vacuum distillation cut of Altamont crude identified as ALT 180 R. This compares to the same sample run using a direct insertion probe. The probe field ionization mass spectrum is shown in Figure 4.

CONCLUSIONS
This small dynamic batch inlet offers several clear advantages over the previous type batch inlets. The advantages are the following:
1. Turnaround time is orders of magnitude shorter.
2. Sample size is up to 1/100th of that used previously.
3. Because of the above and continuous carrier gas sweep, the MS source, transfer line, and bulb stay clean longer.
4. Low cost, easy maintenance.
CRUDE OIL CHARACTERIZATION BY DIRECT SAMPLING ION TRAP MASS SPECTROMETRY*

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Oak Ridge National Laboratory, Analytical Chemistry Division,
P. O. Box 2008, Oak Ridge, TN 37831-6120

The purpose of this work was to evaluate the potential of direct sampling ion trap mass spectrometry (ITMS) techniques for the rapid differentiation of crude oils. Twelve crude oils (Table 1) from five Middle Eastern countries were examined in this study by gas chromatography (GC) with flame ionization detection, combined gas chromatography/mass spectrometry (GC/MS) and direct sampling ITMS. All crudes were obtained from the National Institute for Petroleum and Energy Research, Bartlesville, OK. The gas chromatographic profiles (requiring 50 minutes analysis time) of the twelve crudes exhibited predominant aliphatic hydrocarbons (paraffins), as well as many minor components, and were qualitatively quite similar, differing primarily in the abundance of the paraffins. Although small differences in the minor components were observed in some cases, the ability to differentiate the oils based only on this information was very difficult.

To selectively examine the non-paraffinic components of these crudes, methanol chemical ionization (CI) mass spectrometry was evaluated. Methanol CI permitted selective protonation of the alkyl aromatic hydrocarbons, alkenes, and heteroatom-containing compounds present in the crudes, while eliminating ionization of the more abundant paraffins. The resulting mass spectra for crudes under these conditions consisted primarily of C7- to C9-substituted benzenes and C9- to C14-substituted naphthalenes. Selected ion chromatograms revealed differences in the relative and absolute abundances of these components in the various oil samples, but the ion signal levels were quite low in most cases and difficult to reproducibly quantitate. In addition, this chromatographic-based technique required approximately 30 minutes analysis time.

Previous research in our laboratory has demonstrated the capability of detecting and quantitatively measuring volatile components in a solution by purging the analytes directly into an ITMS without chromatographic separation. To adapt this approach to the differentiation of crude oils, aliquots of the oils were diluted into a methylene chloride/methanol mixture. The resulting solution was spiked into distilled water. The volatile components from the oil samples were then purged from the solution with helium directly into the transfer line of the ITMS, with no chromatographic separation. The effluent entering the ITMS was ionized using water chemical ionization, using the water purged from the sample as the CI reagent. As observed previously with methanol CI, this allowed selective ionization of alkyl aromatics and heteroatom-containing compounds, but not the more predominant paraffins. The volatile components could then be characterized in approximately 3 minutes, compared to 30 or more minutes for the chromatographic based techniques.

From the resulting mass spectra, relative ion abundances for the alkyl aromatic hydrocarbons (ions with m/z 93, 107, 121, 135, and 149) from the 12 crude oils were normalized to the signal from ions with m/z 107, which was usually the most abundant ion. Replicate analyses demonstrated good precision in the relative ion abundances, as shown in Table 1. From these data, it may be seen that differentiation of many of the crude oils is possible using these ion ratios. In two cases, 90SPR53 and 75047, the relative ion ratios were observed to be similar, however the absolute abundances of the alkyl aromatics varied considerably and could be used to distinguish these oils. In order to quantitatively compare absolute ion abundances more readily, an internal standard (such as deuterated xylene) could be added to the oil samples prior to analysis. It should be noted that the alkyl aromatics used in these initial investigations may not be the best components for identification purposes because weathering of the crudes can change the relative and absolute abundances of these volatile compounds. Instead of using direct purge into the ITMS, thermal desorption ITMS could be used to examine the higher molecular weight constituents of the oils (e.g., naphthalenes, phenanthrenes, and heteroatom-containing compounds), which should be less susceptible to weathering effects since they are less volatile. Previous work in our laboratory has demonstrated that thermal desorption ITMS, another direct sampling ITMS technique, also offers advantages of speed and sensitivity for the detection of targeted compounds in mixtures, such as oils.

Table 1. Ion abundance ratios from twelve Middle Eastern crudes determined by direct sampling ion trap mass spectrometry.

<table>
<thead>
<tr>
<th>Oil Sample ID*</th>
<th>m/z 121/107</th>
<th>m/z 135/107</th>
<th>m/z 149/107</th>
<th>m/z 93/107</th>
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</thead>
<tbody>
<tr>
<td>90SPR53*</td>
<td>0.90 ± 0.02</td>
<td>0.49 ± 0.03</td>
<td>0.09 ± 0.01</td>
<td>0.58 ± 0.02</td>
</tr>
<tr>
<td>75047*</td>
<td>0.89 ± 0.02</td>
<td>0.51 ± 0.02</td>
<td>0.15 ± 0.01</td>
<td>0.68 ± 0.05</td>
</tr>
<tr>
<td>70007*</td>
<td>0.91 ± 0.03</td>
<td>0.51 ± 0.02</td>
<td>0.11 ± 0.006</td>
<td>0.62 ± 0.05</td>
</tr>
<tr>
<td>67066*</td>
<td>0.91 ± 0.03</td>
<td>0.61 ± 0.01</td>
<td>0.15 ± 0.004</td>
<td>0.71 ± 0.05</td>
</tr>
<tr>
<td>70070*</td>
<td>0.85 ± 0.002</td>
<td>0.50 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.75 ± 0.003</td>
</tr>
<tr>
<td>69068*</td>
<td>0.87 ± 0.02</td>
<td>0.52 ± 0.01</td>
<td>0.12 ± 0.02</td>
<td>0.66 ± 0.04</td>
</tr>
<tr>
<td>72022*</td>
<td>1.23 ± 0.02</td>
<td>0.85 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>0.73 ± 0.001</td>
</tr>
<tr>
<td>74023***</td>
<td>0.82 ± 0.02</td>
<td>0.47 ± 0.02</td>
<td>0.11 ± 0.001</td>
<td>0.68 ± 0.03</td>
</tr>
<tr>
<td>69080***</td>
<td>0.80 ± 0.01</td>
<td>0.45 ± 0.03</td>
<td>0.10 ± 0.002</td>
<td>0.73 ± 0.01</td>
</tr>
<tr>
<td>69085*</td>
<td>0.76 ± 0.01</td>
<td>0.35 ± 0.01</td>
<td>0.07 ± 0.004</td>
<td>0.74 ± 0.02</td>
</tr>
<tr>
<td>69086*</td>
<td>0.72 ± 0.01</td>
<td>0.40 ± 0.02</td>
<td>0.11 ± 0.03</td>
<td>0.87 ± 0.10</td>
</tr>
<tr>
<td>70113*</td>
<td>0.83 ± 0.001</td>
<td>0.42 ± 0.02</td>
<td>0.10 ± 0.02</td>
<td>0.68 ± 0.02</td>
</tr>
</tbody>
</table>

Country of Origin:

*Iraq
*Iran
*United Arab Emirates
*Saudi Arabia
*Kuwait

*Identities assigned by National Institute for Petroleum and Energy Research, Bartlesville, OK.

**Oil from same field, but one sampled in 1974 and other in 1969.
Low ppm Detection of Stigmastanes and Propylcholestanes in Biomarkers Analysis Using GC/MS/MS

Siu T. Teng*, Finnigan MAT, San Jose, CA; Paul Lipson and J. Michael Moldowan, Chevron Oil Field Research Company, Richmond, CA

Introduction

The use of biomarker analysis in petroleum exploration has been established during the last two decades [1]. Recent developments in tandem quadrupole mass spectrometry regarding MS/MS ion transmission, sensitivity, and reproducibility have made the application of these advanced GC/MS/MS techniques suitable for biomarker analysis in petroleum exploration [2].

Experimental

All experiments used a Finnigan MAT TSQ 700 triple stage quadrupole mass spectrometer interfaced to a Varian 3400 GC.

GC conditions

A J&W bonded phase methyl silicone (DB 1) fused-silica capillary column, 60 meters long, 0.25 mm I.D., 0.25 mm film thickness was connected directly to the ion source through a transfer line maintained at 280°C. The temperature program was 2°C/min. from 150°C to 300°C. Helium carrier gas was used at an inlet pressure of 16 psi which gave a linear velocity of 19 cm/s measured at 300°C isothermal.

Selected Reaction Monitoring (SRM) in Parent MS/MS mode was performed using Argon collision gas at 0.8 mTorr as indicated at the meter, with no correction for gas type. Electrometer gain was 10E-8 A/V and electron multiplier voltage 1600 Volts. Four homologous ions, the parents of m/z 217, and four daughters of m/z 414.4 were monitored in an automated multi-mass experiment. The SRM mode used a scan time of 100 msec and a mass window of 0.3 daltons centered on the ion mass. A collision energy of -14 eV was the best of a series from -8 to -20 eV at m/z 414.4. The sample volume was 0.1 μl of undiluted material. All experiments were automated using the ICL procedure which also controlled the GC parameters.

A petroleum saturate fraction with known concentrations of steranes and diasteranes served as a standard. This was examined at 5, 10, and 15 kV conversion dynode voltages. The other experiments were conducted using 15 kV conversion dynode voltage.

Results and Discussion

Fig. 1 shows the mass chromatograms of the 5 parent ions of m/z 217.2 for the standard run. The peaks are numbered to correspond to the steranes listed in Table 1. The separation of the mass chromatograms from the multi-mass experimental data was done using the Interactive Chemical Information System (ICIS) software. Fig. 2 shows the mass chromatograms of 5 of the homologous parent ions of m/z 217.2 in the sample run. Fig. 2 also shows m/z 414.4 as the parent of m/z 231.2, 290.5, and 304.3. Fig. 3 shows the mass chromatograms of the sample run with numbered peaks for comparison with Table 1. The quantitation estimate for the sample gave values ranging from 0.1 to 10 ppm as summarized in Table 2. The very weak mass chromatogram (peak near noise level) of the MS/MS transition of m/z 414.4 to m/z 231.2 (Fig. 2) shows methylated C-30 sterane isomers to be present at or below the detection limit of this instrument. Fig. 2 also demonstrates the typical dynamic range of 3 orders of magnitude in the SRM mode of the system, based on the scale ranges shown at the right of the chromatograms.

References

Table 1

<table>
<thead>
<tr>
<th>Peak</th>
<th>Parents of m/z 217 Steranes</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td>5β-Cholane</td>
<td>330</td>
</tr>
<tr>
<td>06.</td>
<td>5α,14α,17α-Cholestan 20S</td>
<td>372</td>
</tr>
<tr>
<td>07.</td>
<td>5α,14β,17β-Cholestane 20R</td>
<td>372</td>
</tr>
<tr>
<td>08.</td>
<td>5α,14β,17β-Cholestan 20S</td>
<td>372</td>
</tr>
<tr>
<td>09.</td>
<td>5α,14β,17α-Cholestane 20R</td>
<td>372</td>
</tr>
<tr>
<td>10.</td>
<td>5α,14α,17α-Ergostane 20S</td>
<td>386</td>
</tr>
<tr>
<td>11.</td>
<td>5α,14β,17β-Ergostane 20R</td>
<td>386</td>
</tr>
<tr>
<td>12.</td>
<td>5α,14β,17β-Ergostane 20S</td>
<td>386</td>
</tr>
<tr>
<td>13.</td>
<td>5α,14α,17α-Ergostane 20R</td>
<td>386</td>
</tr>
</tbody>
</table>

Fig. 1.

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>5α, 14α, 17α-Sugmasane 20S</td>
<td>112 ppm</td>
<td>1 - 4 ppm</td>
</tr>
<tr>
<td>5α, 14β, 17β-Sugmasane 20R</td>
<td>104 ppm</td>
<td>1 - 4 ppm</td>
</tr>
<tr>
<td>5α, 14α, 17α-Sugmasane 20S</td>
<td>104 ppm</td>
<td>1 - 4 ppm</td>
</tr>
<tr>
<td>5α, 14β, 17β-Sugmasane 20R</td>
<td>104 ppm</td>
<td>1 - 4 ppm</td>
</tr>
<tr>
<td>13α, 17α-Diacholestane 20S</td>
<td>79 ppm</td>
<td>5-10 ppm</td>
</tr>
<tr>
<td>13b, 17α-Diacholestane 20S</td>
<td>40 ppm</td>
<td>2 - 6 ppm</td>
</tr>
<tr>
<td>13α, 17α-Diacholestane 20R</td>
<td>40 ppm</td>
<td>2 - 6 ppm</td>
</tr>
<tr>
<td>13b, 17α-Dia-24-n-Propylcholestane 20S</td>
<td>12 ppm</td>
<td>1-4 ppm</td>
</tr>
<tr>
<td>13b, 17α-Dia-24-n-Propylcholestane 20R</td>
<td>12 ppm</td>
<td>1-4 ppm</td>
</tr>
<tr>
<td>5α, 14α, 17α, 24-n-Propylcholestane 20S</td>
<td>16 ppm</td>
<td>0.1-0.5 ppm</td>
</tr>
<tr>
<td>5α, 14β, 17β, 24-n-Propylcholestane 20R+20S</td>
<td>70 ppm</td>
<td>0.5 - 2 ppm</td>
</tr>
<tr>
<td>5α, 14α, 17α, 24-n-Propylcholestane 20R</td>
<td>19 ppm</td>
<td>0.1-0.5 ppm</td>
</tr>
<tr>
<td>13α, 17α-dia-24-n-Propylcholestane 20S</td>
<td>12 ppm</td>
<td>1-4 ppm</td>
</tr>
<tr>
<td>13b, 17α-dia-24-n-Propylcholestane 20R</td>
<td>10 ppm</td>
<td>1-4 ppm</td>
</tr>
</tbody>
</table>

Fig. 2.

Fig. 3.
The discovery of low levels of organoarsenic compounds in natural gas from certain production fields has now required developing methods to scrub these compounds from the gas before it is used as industrial and domestic fuel. Engineering the most effective scrubbing method requires knowing which arsenic compounds are present. We have employed low temperature capillary gas chromatography-mass spectrometry (gc-ms) combined with various enrichment techniques to identify the molecular form(s) in which the arsenic is found.

Instrumentation: All gc-ms experiments were performed on a Hewlett-Packard 5995c quadrupole instrument, equipped with a DB-5 (J&W Scientific) bonded phase column, 30 m x 0.32 mm x 1.0 μm film. A H-P Pascal ChemStation datasystem was coupled to the gc-ms. Standard autotuning was performed using perfluorotributylamine calibrant. The standard chromatographic temperature program adopted was: initial temperature -50°C, programmed at 5°C/minute to 60°C, to give a 22 minute run. Split injections of from 0.1 to 1.0 ml (at ambient temperature and pressure) at 15:1 to 30:1 split ratios were made into a 300°C injection port with a valved gas-tight syringe. The mass spectrometer was scanned repetitively from m/z 15 to m/z 550 at appx. 0.7 scans/second. Subambient temperatures in the gc oven were achieved by using liquid nitrogen coupled to and controlled by the datasystem and gc-ms hardware.

Compounds: Hydrocarbon retention indices were established using a calibration mixture of appx. 0.1% (by volume) CH₄ through C₆H₁₄ in helium (Scott Specialty Gases) for identifying unknowns. Both methylarsine and dimethylarsine were synthesized in order to measure retention indices[1] and record authentic mass spectra. They were prepared by reduction of, respectively, methanearsenic acid and dimethylarsenic acid with NaBH₄ in acidic solution. Trimethylarsine, available from our own reference collection, was also run. Natural gas sampled from the laboratory jet (Lone Star Gas Co.) was used as an additional reference material.

Reference Spectra: Spectra of methylarsine, dimethylarsine and trimethylarsine are shown in the accompanying figures. The first two compounds are not present in the NIST/EPA/MSDC mass spectral database. Our spectrum of trimethylarsine is at variance with the one found in the NIST database. The database spectrum shows m/z 77 as base peak, m/z 75 at appx. 95%, followed by m/z 103, m/z 105 and m/z 120 (M⁺) at 80%, 66% and 64%, respectively. No information on origins or conditions for recording the reference spectrum was available to us. Our results suggest the m/z 75, 76 and 77 peaks in the NIST reference spectrum are extraneous, but that the abundance ratios of the remaining peaks to each other are correct. Chromatographic retention indices for these three compounds on the DB-5 column are: methylarsine, 404; dimethylarsine, 504; trimethylarsine, 553.

Direct Analysis of Gas: Direct injection of laboratory gas and the hydrocarbon reference mixture demonstrated that, while separation of methane and ethane was less than optimal, the rest of the normal hydrocarbons were well spaced along the retention time axis. However, injection of a natural gas sample, determined by total arsenic analysis[2] to contain 650±40 ng arsenic per liter, demonstrated the levels of arsenic compounds present to be undetectable by such a simple approach. Two procedures were tried in order to selectively enrich organoarsenic compounds over the large amounts of hydrocarbons.

Enrichment Procedures: The first procedure used cryogenic trapping to concentrate the less volatile components of natural gas. The boiling points of the three model organoarsines are 2°C, 36°C and 52°C[3]. These compounds should be cryogenically trapped using frozen mixtures of dry ice or liquid nitrogen and various solvents[4]. Measured volumes of arsenic-containing natural gas were passed through an appropriately flushed cold trap, and the cold trap was sampled for gc-ms analysis. While very clearly enriching the less volatile hydrocarbon components of the natural gas, this procedure failed to enrich for organoarsenic compounds.

The second enrichment procedure consisted of oxidizing the organoarsines from the +3 to the +5 oxidation state and trapping them by bubbling gas through a 2.5M HNO₃ solution. After bubbling the appropriate amount of gas through the acid solution, NaBH₄ was added to reduce the dissolved organoarsine oxides back to their parent organoarsines. The regenerated organoarsines were then purged from solution with helium, cryogenically trapped, and sampled for gc-ms analysis.

Results & Discussion: We do not know at this time why the cryotrapping technique did not collect organoarsenic compounds. The oxidative trapping technique succeeded in collecting a very small amount of methylarsine. The selected ion chromatograms of m/z 57 (hydrocarbon fragment) and m/z 92 (M⁺ of methylarsine) shown below indicate a very small amount of methylarsine on the trailing side of the large n-butane peak. The
presence of methylarsine was only detected by reconstructing selected ion chromatograms with the datasystem software. The correct intensity ratios of the important ions in the molecular ion region are present in this small peak, and it occurs at the correct retention index determined for methylarsine.

We have direct gc-ms evidence only for the presence of methylarsine. Indirect evidence exists, however, for the presence of other compounds from total arsenic determinations[2,5], and from identification of various mixed methyl and ethyl arsine sulfides found in solids collected from gas pipelines and fouled pipeline valves. Examination of such solids by electron ionization direct insertion probe experiments clearly showed the mass spectral details expected for such compounds, and matched our observations on materials synthesized in the laboratory[6].

While low temperature gc-ms provides the structural specificity to unambiguously identify organoarsine species in natural gas, a reliable enrichment scheme must be developed. Our attention will be focused here in future work.

NITRIC OXIDE CHEMICAL IONIZATION GC/MS IN THE STUDY OF THE SOURCE OF LIGHT CYCLOALKANES
IN PETROLEUM  T. P. Fan, F. D. Mango,  Shell Development Company Bellaire Research Center, Houston, Texas 77025

A theory developed by Thompson suggested that the polycycloalkanes are less thermally
stable than open-chain structures and thus decompose earlier during catagenesis. He
proposed that the heavier polycyclic hydrocarbons, such as steranes and triterpanes, are
the precursors to the lighter cycloalkanes through thermal decomposition. Mango
disagrees with the above theory in various aspects. He indicates that theoretically,
more energy is required to thermally crack ring structures than to crack open chain
configurations. An alternative explanation is that the lighter cycloalkanes are formed
through a steady-state catalytic process similar to that of the isoheptanes.

Thompson's theory was tested experimentally by thermally decompose a mixture of
cholestane (Figure 1) and octadecane under a simulated subsurface temperature of 330°C
for 4 weeks. The resulting solution was submitted for GC/MS identification. The
components in the C5-C8 region are expected to be a mixture of alkanes and olefins,
derived from thermal cracking of octadecane and from cholestane side-chain, and
possibly the cycloalkanes produced from cholestane. It is the goal of this experiment
to determine whether C₅ to C₈ cycloalkanes are present. However they are difficult to
distinguish from olefins by EI due to spectral similarity, also not all the olefin
isomer standards are available for identification by their retention times.

The identification was successfully accomplished using nitric oxide chemical ionization
(NOCI) GC/MS. The NOCI GC/MS technique employs Townsend discharge for producing
reagent gas ions NO⁺ in the ion source. Interaction of alkanes and cycloalkanes with
NO⁺ results in exclusively (M-H)⁻ ions. While interaction of olefins with NO⁺ produces
(M+NO)⁺ ions and characteristic even mass fragment ions (corresponding to (MNO-C₅H₁₀)⁻)
ions at m/z 86 (C₅H₉NO⁻), 100 (C₆H₈NO⁻), and other alkyl homologues. These spectral
characteristics provide a clear distinction between cycloalkanes and olefins.

Figures 2(a) and 2(b) shows the EI GC/MS chromatogram of the C5 to C8 products and the
sample spiked with seven standard compounds respectively. Figure 2(b) demonstrates that
several components closely eluted with spiked standards, including two of the major
products peaks 11 and 19, and their EI spectrum are very similar. Using NOCI, peaks 11
and 19 are unambiguously identified as 1-heptene and 1-octene respectively, as
de decomposition product from octadecane. Figures 3 and 4 show the clear distinction of
olefins and cyclic components in the ion chromatograms (M-H)⁻, (M+NO)⁺ covering the
retention regions I and II indicated in Figure 2(b). The NOCI spectra of 1-octene and
1,2-dimethyl cyclohexane are shown in Figures 5 and 6.

Based on NOCI GC/MS spectra, all the components within the C5-C8 region were
successfully identified (Figure 7). Approximately equal amounts of paraffins and
olefins were found. They contributed more than 90% of the product in this range with
equal contribution from the side chain of cholestane and octadecane. Only
insignificant amounts of cyclic compounds were present. The findings here provided
positive evidence for cracking of the cholestane side-chain before the opening of
cyclic ring structure under thermal decomposition conditions.

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CHOLESTANE THERMAL DECOMPOSITION
C5-C8 PRODUCTS

Figure 1

Expanded Region I Sample - Standard

Figure 2

NORMALIZED INTENSITY

RETENTION TIME (MINUTES)

Figure 3

Expanded Region II Sample - Standard

Figure 4

NOCI Spectrum of 1-Octene (Peak # 18)

Figure 5

NOCI Spectrum of 12-Dimethyl Cyclohexane Standard

Figure 6

Figure 7
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ANALYSIS OF FOSSIL-FUEL FRACTIONS BY ON-LINE COUPLED MICROCOLUMN HPLC CAPILLARY GC-MS

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* Current Address: S. C. Johnson and Son, Inc., Racine, WI 53403

INTRODUCTION: Multidimensional chromatography (e.g., HPLC-GC) allows one to take advantage of the peak capacity [1] (number of completely resolved analytes) in each technique; because, the peak capacity of the combined technique is the product of the individual techniques [2]. A micro HPLC-GC-MS system was developed and applied to the analysis of solvent-refined coal (SRC-II).

EXPERIMENTAL: An ISCO syringe pump supplied mobile phase (10% heptane in pentane) at a flow rate of 10 μL/min to a Rheodyne 7520 injector (0.2 μL). A 0.32 mm ID x 300 mm fused-silica column, slurry packed with 5-μm 2,4-dinitrophenylmercaptopyrrol silica [3], was connected between the injector and a 0.065-mm ID fused-silica transfer line. The column ends were fitted with 0.32 mm OD x 2 mm Teflon® frits. The column effluent was monitored by UV detection through a window burned in the polyimide coating of the transfer line.

The transfer line was attached to a Valco ten-port valve (0.010” ID ports). A 50-μL sample loop and a7.6-μL flush loop were attached to the valve. Helium GC carrier gas entered the valve at a pressure of 15 PSI and exited via a 0.32 mm ID x 5 m retention gap connected to the GC column.

An HP-5890A GC was interfaced to a Finnigan 4000 with an SGE open-split interface. A 0.25 mm ID x 30 m J&W DB-5 column was connected between the retention gap and the open-split interface. The operation of the open-split interface was modified slightly to allow it to function as a high-volume solvent vent during concurrent cosolvent evaporation.

SRC-II was diluted to 4% with hexane, filtered with a 0.45-μm membrane filter, and repetitively injected into the HPLC system. While HPLC fractions were collected in the sample loop (as shown) the flush loop was filled with pentane. After collection the valve was switched. An interval timer switched the open-split interface to vent for a specified period of time (25-45 minutes). During this interval of concurrent-cosolvent evaporation, the GC oven was maintained at 65°C. At the end of the timed interval the open-split interface reverted to normal flow and the GC temperature program and data acquisition were actuated.

RESULTS: The fractions were characterized as: 1) alkyl benzenes, indenes and tetrains, 2) alkyl naphthalenes, 3) fluorenes, dibenzofurans and 4) phenanthrene. Generally biphenyls elute with naphthalenes; however, as observed here and in other analyses, ortho-substituted biphenyls elute earlier.

3. Welch and Hoffman HRC&CC 1986, 9, 417.

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USE OF HYDROCARBON HOMOLOGUES AS INTERNAL MASS CALIBRANTS FOR ACCURATE MASS MEASUREMENTS

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Exxon Research and Engineering Co.
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Petroleum and synthetic fuel fractions are very complex mixtures which contain thousands of components. Accurate mass measurement of a wide range of components using high resolution mass spectrometry (HRMS) is a common practice in the petroleum industry for the determination of compound type distributions. The petroleum distillate is usually separated into fractions by chromatography either on-line or off-line, and then each fraction is subjected to mass spectrometric analysis. Since accurate mass measurement using a double focusing mass spectrometer requires the presence of both the mass calibrant and the sample, the calibrants are chosen not to contain components present in the sample. For example, in high voltage (70 V) electron-impact ionization (EI) perfluorokerosene (PFK) is chosen for HRMS accurate measurement of hydrocarbons because (1) no fluorine-containing compounds are present in petroleum and synthetic fuels, (2) PFK components cover a wide mass range, and (3) there are reasonably small mass differences between adjacent PFK peaks for calibration.

When other ionization methods are used, PFK becomes an unsuitable calibrant and other synthetic blends are usually needed. One example is the use of a blend of halogenated aromatic compounds for low voltage EI high resolution MS measurements of hydrocarbon mixtures because PFK is not ionized under low voltage EI conditions.

There are several disadvantages in using external mass calibrants (calibration standards): (1) various calibrants need to be prepared for different ionization conditions, (2) the calibrant dilutes the sample, therefore reduces the sample detectability, (3) a proper ratio of calibrant to sample needs to be maintained so that calibrant peaks can be recognized, (4) the calibration range of the standard may not cover the whole range of the sample components and (5) the spacing between calibrant components is too wide to provide accurate measurements for sample components in between.

To eliminate these difficulties, we have developed a new calibration procedure using the major homologous series present in the sample mixture as internal mass calibrants for accurate mass measurement. An external standard is used for preliminary confirmation of the most abundant series. Once the series is identified, the members of the whole series are used as calibrant peaks. The presence of the external standard is no longer needed. Since other series are present in about the same mass range as the calibrant series and the spacing between the members of the calibrant series is 14 mass units apart, more accurate measurements of other series are obtained than using external standard alone. A computer program has been developed on the VG OPUS data system for quickly editing the reference mass reference files of homologous series.

Figures 1 and 2 show that different results were obtained from calibration using external and internal standards. In the examples shown, a blend of halogenated aromatics was used as an external calibrant for low voltage EI high resolution MS measurement. Due to low volatility, no components with molecular weights greater than 500 could be introduced into the ion source via calibrant entrance of the mass spectrometer. As a result, shown in Figure 1, all of the heavy components were missing after calibration. However, the whole of both series was identified when an internal standard was used to calibrate the same two series, as shown in Figure 2.
The internal standard can also be combined with the external standard to further confirm the identification of internal standard series. If the internal standard series is correctly identified, all of the calibrant peaks will be included in the final calibration table, as shown in Figure 3. If the series has been mis-assigned, then many calibrant peaks will be missed in the calibration table, as shown in Figure 4.

In conclusion, the use of homologous series as internal calibrants facilitates mass calibration and provides more accurate mass measurements. The internal calibration procedure is found particularly useful for on-line LC/MS high resolution MS analysis of high boiling hydrocarbon mixtures.

**Figure 1**

All the heavy components are missing using an external standard.

**Figure 2**

Entire series are identified using an external standard.

**Figure 3**

Combination with external calibrant peaks confirms the identification of internal calibrant series.

**Figure 4**

Misidentification of internal calibrant series is recognized by a combination with external calibrant peaks.
Characterisation of Crude Oils by Field Ionisation Mass Spectrometry using a Retractable All Glass Heated Inlet System (AGHIS) for Sample Introduction.

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Introduction

Field ionisation mass spectrometry (FIMS) is an excellent method for analysing complex mixtures since FIMS yields practically fragment-free molecular ion spectra. This technique is therefore ideally suited to the characterisation of crude oils and crude oil fractions since the molecular weight distribution can be directly determined and the contribution from all the components evaluated.

This study involves the comparison of data obtained from two crude oil fractions by 70eV El (which has traditionally been used to evaluate oil fractions on the basis of selected fragment ions) low eV El and FI techniques.

All the samples were introduced through a retractable All Glass Heated Inlet System (AGHIS).

Experimental

The comparative study of the three different ionisation techniques were performed using a VG AutoSpec mass spectrometer. Sample was introduced through the ACHIS heated to between 300 and 350°C. The ACHIS is mounted in a bellows mechanism and can be moved within the source housing by means of a scissor jack. This allows the inlet to be used with either the EI or FI sources. The new open construction of the VG FI/FD source, allows the tip of the ACHIS jet to be positioned 1 to 2mm away from the FI emitter wire, increasing sensitivity. This open construction also reduces the surface area of the source, minimising memory effects and background problems.

The spectra shown FIG 1 & 2 (SAMPLE A, SAMPLE B) are of two crude oil fractions run using the retractable ACHIS, comparing 70eV EI, low eV EI and FI data in each case.

Discussion

Differences in the amount of fragmentation and the relative response of peaks at different masses are evident from the results shown. Analysis using 70eV EI ionisation produces good quality reproducible data. The high degree of fragmentation, however, limits the amount of information and its accuracy. The analysis of this data is based on the summation of characteristic fragment ions to indirectly determine the relative concentration of the component hydrocarbon types present.

With low eV EI conditions this fragmentation is significantly reduced but with an associated drop in intensity. Much more molecular ion information is present, however some shift to lower mass fragment ions is visible.

FI yields fragment free molecular ion information with a high degree of reproducibility. The oil fractions can be easily characterised from the relative intensities of the molecular ions from each z-number series.

As the new open design of the AutoSpec FI/FD source allows the ACHIS to be close coupled to the emitter wire, sensitivity is very good. This allows good quality, reproducible, high resolution data to be obtained in FI.
FT-IR studies of absorbed probe molecules are currently being used to elucidate the acid-base properties of metal oxide supports. Such studies for the reactions of alcohols chemisorbed on gamma alumina by Knozinger et al. (1,2) led them to suggest that short chain alcohols chemisorb on alumina to form two species: an alkoxide, which is formed by the dissociative chemisorption of the alcohol on an acid-base site; and a coordinated species, formed by the chemisorption of the alcohol onto coordinatively unsaturated aluminum ions (Lewis acid sites).

We report here some preliminary results of the application of a novel mass spectrometric technique to study the adsorption sites and subsequent reactions of short chain alcohols on calcined gamma alumina. This technique involves using the direct temperature-controlled probe as the reaction chamber to perform in situ mass spectrometry with isotopically labelled alcohols. We have found that this technique provides both greater sensitivity and a more precise experimental record for the various catalytic reactions than conventional techniques such as temperature programmed desorption and the use of ultra high vacuum systems.

The analyses of catalyzed reactions of both 1-propanol and of ethanol-18O will be used to illustrate the technique. Reconstructed partial ion chromatograms of the reaction of 1-propanol on alumina are shown in Figure 1. Initially, at a relatively low temperature, only desorption of physisorbed water and alcohol is observed as evidenced by the selected ion traces of m/z 17, 18 and m/z 59, 60. As the temperature increases, two products are observed: the lower temperature product on the m/z 73, 102 trace can be attributed to dipropyl ether; the higher temperature product is the propene which gives a signal at m/z 41, 42. These products are the result of dehydration reactions in which water is produced. Evidence for the coproduction of water is seen in the m/z 17, 18 ion trace, which shows inflections corresponding to the formation of the ether and the alkene.

Ether formation is a bimolecular reaction and requires that the alkoxide groups are on adjacent sites while the alkene is formed by the unimolecular elimination of water from an isolated alkoxide species. This accounts for the ether being formed initially, and only when all adjacent alkoxide species have been depleted does the alkene formation reaction begin to occur.

To learn more about the nature of the alkoxide species giving rise to these products, similar reactions were carried out using ethanol-18O (Figure 2). In addition to the elimination of the two different water species (m/z 17, 18 and m/z 19, 20) and the production of ethene (m/z 26, 28), this reaction produces two isotopically different ether products as evidenced by ion chromatograms for m/z 59, 74 and m/z 61, 76. These are molecular ions which can be attributed to CH₃CH₂OCH₂CH₃ and CH₃CH₂OCH₂CH₂ respectively. The only explanation for formation of these two ether products is that they originate from two different ethoxide species, one incorporating an O-18, the other O-16.
The acid and base sites on the surface of alumina can be pictured according to scheme 2 (3).

\[ \text{OH} \quad \text{heat} \quad \text{(OH)}^+ \quad \text{O}^+ \]
\[ \text{Al} / \text{Al} \quad \rightarrow \quad \text{Al}^+ / \text{Al} \quad \text{or} \quad \text{Al} / \text{Al} \]
\[ \text{O} / \text{O} \quad \text{O} \quad \text{O} \]

The presence of the two alkoxide species can only be explained by considering that the ethanol reacts with the Lewis acid site, as suggested by Knozinger (1,2), and also reacts with the oxide of the basic site. These two mechanisms are shown in Scheme 3.

\[ \text{Et}^{18}\text{O}-\text{H} \quad \text{Et}^{18}\text{O}-\text{H} \quad \text{Et} \quad \text{Et} \]
\[ \downarrow \quad 18\text{O} \quad \downarrow \quad 18\text{O} \quad 16\text{O} \quad + \quad \text{H}_2^{18}\text{O} \]
\[ \text{Al} / \text{Al} \quad \rightarrow \quad \text{Al} / \text{Al} \]
\[ \text{O} / \text{O} \quad \text{O} \quad \text{O} \]

In conclusion, using $^{18}$O-ethanol we have shown that ethanol reacts with the alumina surface to produce two alkoxide species. This novel technique is currently being applied more extensively to other catalytic systems.

Distillation fractions of a hydrotreated diesel fuel were analyzed for aromatic and saturate types by mass spectrometry using modified versions of the ASTM methods for hydrocarbon types in low olefinic gasoline (D2789) and in middle distillates (D2425). The diesel fuel had been distilled into nominal 10% volume fraction cuts. The first three boiling fractions were in the gasoline range and were, therefore, analyzed for hydrocarbon types using the low olefinic gasoline method. The seven higher boiling fractions were analyzed by the middle distillate method. These diesel range distillation cuts required separation into saturate and aromatic fractions prior to mass spectrometry type analysis. This was accomplished by open column chromatography using a modified version of ASTM D2549, in which only pentane and diethyl ether are used as elution solvents. Both type analysis methods were also modified by substituting a quadrupole mass spectrometer for a magnetic sector mass spectrometer. These data were required to determine whether fractionation would help in producing a diesel product which would meet the new California air control standards.

The mass spectral analysis showed that the total aromatic hydrocarbon content increased in the higher boiling fractions, while the saturate hydrocarbons generally decrease as demonstrated by figure 1. A more detailed review of these data showed that the polynuclear aromatic compounds significantly increased in the higher fractions, while the mononuclear aromatic compounds such as alkylbenzene and alkylindane actually decreased as shown in figure 2. These results are significant because the naphthalene, biphenyl, fluorene, and phenanthrene content, which make up the major polynuclear aromatic components, have been shown to correlate with diesel particulate emissions.

The individual saturate hydrocarbon types also show diverging trends with boiling range as seen in figure 3. While the paraffinic content increased as the boiling temperature increased, the one ring saturates decreased substantially. The two ring saturates remained relatively constant over the entire range and the higher ring saturates showed moderate increases at the upper part of the range.

The results of this study show that the individual hydrocarbon composition varies greatly along the boiling range of the hydrotreated diesel fuel. Since the polynuclear aromatic compounds for the diesel sample are concentrated in the higher boiling ranges, controlled distillation may be used to remove great proportions of these components which are known to be strong contributors to diesel particulate emissions.
Preliminary results indicated plasma desorption mass spectrometry (PDMS) might be an ideal ionization method for analyzing metalloporphyrins in petroleum and shale oil samples. Conventional analysis of metalloporphyrin content involves separation of the polar porphyrin components from the hydrocarbon constituents followed by ultraviolet absorption spectroscopic analysis. Analysis of metallogeoporphyrins provides a means for obtaining relative maturation values of petroleum and shale oil crudes. This paper describes the background studies necessary for investigation of the potential of PDMS in metallogeoporphyrin analysis.

A Bioion 20 (Applied Biosystems AB, Uppsala, Sweden) plasma desorption mass spectrometer was used in these studies. The samples were dissolved in a 50/50 tetrahydrofuran/acetone solution prior to electrospraying onto a nitrocellulose-coated mylar target. The samples were inserted into the Bioion sample carousel for mass analysis. The acceleration potential used for these studies was 18000 volts. The ETIO vanadylporphyrin standard was purchased from Midcentury Chemicals (Posen, IL). The purity was checked for significant impurities using isobutane chemical ionization - none were detected.

The quality of the PDMS spectrum for high and low levels of the ETIO vanadylporphyrin standard is shown in Figure 1. As can be seen, there is a significant change in signal-to-noise levels in going from 100 ppm to 1 ppm. Also when higher levels of ETIO vanadylporphyrin are present on the target, fragmentation can be observed. Figure 2 shows the intensity of the PDMS signal of both a 1 ppm and a 10 ppm vanadyl signal over eight successive two-hour intervals. The intensity of the (M+H)+ ion in the last two hour interval remains very strong in both graphs, showing very little decrease in the 10 ppm sample, and a decrease to the 30% level of the intensity of the initial two hour interval in the 1 ppm sample.

With a better understanding of the detection limits achievable in the analysis of a pure vanadyl geoporphyrin using PDMS, the next step was to spike a petroleum sample. The petroleum chosen was a Djeno crude which did not have any detectable metalloporphyrins by the conventional separation/ultraviolet absorption spectroscopic method. Figure 3a shows the PDMS spectrum of the spiked (ETIO vanadylporphyrin - 100 ppm) crude (electrosprayed with 50/50 THF/Acetone) with no sample preparation. Figure 3b is the PDMS spectrum of the same sample after undergoing a simple methanol extraction. Notice the considerable improvement in both intensity and signal-to-noise with this preliminary extraction. This analysis speaks to the detection limits of a metallogeoporphyrin where all of the porphyrin intensity results from one component. This is not the case for a real petroleum sample in which the intensity of the metallogeoporphyrins is spread over a number of different homologs.

Figure 4 shows the PDMS spectrum of a Wilmington crude which has been separated and purified using chromatography and characterized using UV absorption spectroscopy. A homologous series of DPEP vanadylgeoporphyrins can be observed (C_{20-24}) m/z 458 - 584 (C_{24} = m/z 528). These results indicate that PDMS is a viable addition to the methodologies presently being used for the analysis of metallogeoporphyrins. PDMS of the sample obtained from a simple methanol extraction scheme can provide an improved mass spectrum of the ETIO/DPEP metallogeoporphyrins in a petroleum crude oil.

Acknowledgements

The authors wish to thank Professor David Freeman (University of Maryland) for his helpful suggestions and for the oil samples he so generously sent us.

REFERENCES
2. D. Freeman, personal communication.
FIGURE 1.

1 NG

FIGURE 2.

10 NG

FIGURE 3.

A)

B)

FIGURE 4.
SPECTRAL ANALYSIS BASED ON BIPOLAR TIME DOMAIN SAMPLING: A NEW MULTIPLEX METHOD FOR TIME-OF-FLIGHT MASS SPECTROMETRY

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We hereby introduce bipolar sampling as a novel, non-Fourier transform, multiplex technique for sampling and frequency-analyzing a signal consisting of a sum of exponentially damped sinusoids of different frequency. The method is illustrated by a hypothetical toroidal time-of-flight mass spectrometer, in which ionic mass is related to the rotational frequency of pulse-injected analyte ions confined to circulate around the toroid (Figure 1). Time-domain data are generated from the (induced) ion current recorded by each of two annular detector plates placed 180° apart at opposite ends of the torus (Figure 2). If successive maximal voltages from the two detectors are stored alternately as positive and negative data (vs. time) after a pulse of analyte ions is injected into the torus, then bipolar sampling effectively mass-separates ions by recording their passage across a given detector at different times.

Unlike Fourier transform sampling, in which the sum of all of the component frequencies is sampled at equally-spaced time increments, the present bipolar sampling records only the extrema of each (individually periodic) signal to yield a (non-equally spaced) time-domain data set in which each frequency component is detected individually. If analyte ions injected isoenergetically into a torus are constrained to execute circular trajectories, then ions of a given mass-to-charge ratio will travel at constant velocity as a coherent packet. The rotational frequency of each ion packet is given by Eq. 1:

$$v = \sqrt{\frac{qV}{2\pi^2m^2r^2}}$$

in which $v$ is the ion rotational (time-of-flight) frequency (Hz), $q$ is the ionic charge (C), $V$ is the ion accelerating voltage (V), and $r$ is the toroidal radius (m). For $V = 5000$ volts and $r = 0.5$ m, the rotational frequencies for singly charged ions of mass 1 to 100,000 u range between 312.664 kHz and 988.73 Hz, well within the range of commercially available analog-to-digital converters.

Recovery of the corresponding mass spectrum from the time domain data requires (non-FT) sequential pattern recognition of the individual frequency components. Figure 3 shows the decomposition of a computer-generated two-frequency time-domain signal into its individual components by use of a pattern recognition algorithm. For a more complex signal with five frequency components (not shown), bipolar sampling followed by pattern recognition afforded excellent component isolation (unit efficiency). Once each component is isolated, its characteristic parameters are estimated with high accuracy: rotational frequency ($10^{-12}$ relative error), mass-to-charge ratio ($10^{-18}$), amplitude ($10^{-4}$), and phase ($10^{-12}$). Predictably, the effect of Gaussian-distributed time-domain jitter on bipolar sampling was to degrade frequency component isolation/characterization. In summary, bipolar sampling is inherently efficient (2 data samples per cycle), achieves the multiplex advantage of FT analysis, requires minimal digital storage capacity, and yields excellent signal frequency accuracy in "zero-noise" simulations. [Work supported by N.S.F. (CHE-9021058) and The Ohio State University.]
High resolution mass spectra of heavy petroleum and synthetic fuel fractions are difficult to analyze because of the complexity of the sample composition. Each spectrum contains thousands of mass peaks even under low energy electron-impact ionization conditions where fragmentations of parent molecular ions are minimal. Complete identification of peaks at every mass in the spectrum is a complicated and time-consuming task, which can be effectively accomplished only with the help of a mainframe computer. The difficulties in data processing become acute in on-line LC-MS analysis where the high resolution mass spectral data are acquired throughout the LC separation, and data analysis of narrow elution regions are frequently needed.

In our efforts to develop on-line LC-MS techniques for the molecular characterization of heavy hydrocarbons, we realized the necessity of inventing a fast, simple, and reliable method to process the high resolution mass spectral data. We noticed that the "messy" appearance of petroleum mass spectra are mostly attributed to the presence of homologous series and isotopic peaks containing $^{13}$C. If these homologous series could be recognized without examining elemental compositions for every mass, the data reduction would be greatly simplified.

In early 1960's, Kendrick proposed a mass scale based on CH$_2$=14.00000 instead of 12C=12.00000 to express organic mass spectral data (1). The advantage of the Kendrick scale is that all compounds in a homologous series have the same mass defect (Table 1). Therefore, all mass peaks can be sorted and grouped into homologous series according to their unique Kendrick mass defect (KMD). However, using KMD alone to sort out homologous series is difficult if not impossible, because it would require better resolving power and less mass measurement error than current high resolution mass spectrometers can provide. Table 2 show a partial list of compound types commonly observed in hydrocarbon mixtures with KMD between -.1167 and -.1608. The mass differences between adjacent members are usually less than 10 millimass units (mmu) and in some cases, even less than 1 mmu. It is not uncommon for errors in accurate mass measurements to be greater than 5 or even 10 mmu. Apparently, additional steps would be needed before we can use KMD for sorting and grouping of compound types.

The compound types shown in Table 2 are in various nominal mass series, or $Z^*$, where $Z^*$ is defined as the difference of the remainder of nominal mass divided by 14 minus 14, i.e., mod(nominal mass/14) - 14. Table 3 shows a list of compound types with a nominal mass series of -6. The closest adjacent members are 3 mmu. Many of them are greater than 30 mmu. Thus, pre-sorting by nominal mass series facilitates the sorting based on KMD.

Based on these considerations, we developed a multiple sorting technique to process high resolution mass spectral data. Mass peaks with their accurate masses are first sorted by $Z^*$ into 14 groups. Within the same $Z^*$ group, the mass peaks are further sorted by their KMD and grouped into several homologous series. In the final step, the masses of the same homologous series are arranged in sequence to judge the quality of multiple sorting. Identification of compound types is carried out by matching the average value of KMD of the series with the stored KMD values of various compound types in the same $Z^*$ series. The final output of multiple sorting is shown in Figure 1.
The multiple sorting technique has several advantages. Compounds in a homologous series are grouped together. General formula of each group is determined from KHD. This procedure is much faster than determining elemental composition of individual mass peak and then sorting them out into groups. Since average KHD is used for each group, measurement errors associated with individual members of the group are compensated for by statistical averaging, resulting in a more reliable determination of molecular formulae. Carbon number distribution within a series can be easily determined once the core structure is identified. Consequently, a computer program is much simpler in structure and works much faster than conventional data processing programs for high resolution mass spectrometry.

REFERENCE:

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<th>TABLE 1</th>
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FIGURE 1
A COMPUTER OUTPUT OF MULTIPLE SORTING

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ADAPTATION OF A RANDOM INFORMATION PROCESSING PROGRAM FOR THE RAPID MANIPULATION OF GC/MS AND RELATED DATA

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The mass spectrometry professional is confronted with an overwhelming array of data including chemical names, synonyms, Chemical Abstracts Registry Numbers, formulae, mass spectra, instrumental parameters, GC retention indices, literature references and abstracts. He is also required to prepare letters, reports, data sheets and even invoices all in a limited time. The increasing availability of low cost instruments and variety of computers, data sources and software only adds to this problem.

The present paper describes the adaptation of an inexpensive IBM PC-compatible personal information management program (PIM) to help the busy spectroscopist or any scientist to store, retrieve and manipulate such random data with a minimum of effort, thereby improving efficiency. The PIM program allows fields and records to be entered and searched immediately with a minimum number of single-letter commands. Every piece of information is searchable. Since many mass spectroscopists desire relatively small databases of, say, 400 pollutants, 200 steroid derivatives or 150 specialized references and abstracts, rather than many thousands of items, they can be held in memory. This allows searching and retrieval to be almost instantaneous even with a floppy disk system.

A personal overview of the data is maintained during a search by display of a DYNAMIC SEARCH ARRAY (Figure 1). Data sets are represented on screen by small squares which can be short notes or multi-page documents. When a search string is typed, character by character, squares not containing the string disappear from the array to show how many hits remain (Figure 1b). These can be examined at any stage after all or part of the string has been keyed.

![Diagram](image_url)

**Figure 1**: Example of DYNAMIC ARRAY during search. (a) squares represent records of data held in memory, dots represent space available in memory for further records, (b) reduction after search string entry; remaining squares represent those records containing the desired string

The relatively small number of data sets held in memory means that the names of compounds can be rapidly found by typing unusual letter combinations even from the middle of a word. Often name location
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in databases is problematic as the typed name must correspond exactly with that stored. This problem is eliminated.

The method allows, for example, an eight peak index of mass spectra to be extended to larger numbers of peaks. Any number of GC retention indices or other parameters can easily be added. A further novel feature is the addition of graphics characters to give line diagrams. A typical mass spectral record for a derivatized amino acid is shown in Fig. 2.

Figure 2: Example of mass spectral data record for cystine tetra-TMS derivative showing mol. wt. (mw), mol. formula, GC retention index (r), key ions (k), Chemical Abstracts Registry Number (cas), selected ions (m), relative abundances, line diagram mass spectrum together with parent compound details.

Similarly, databases of full literature references and abstracts can be stored and searched easily to retrieve all abstracts on a specific analyte or matrix and/or by a given author. Such data sets can be held in memory even when other programs such as a word-processor or spreadsheet are in use. Instant switching without exiting and instant data transfer between programs is possible, reports can be written and printed out and where a suitable modem is present, stored telephone numbers can be dialled. A single file may be up to 10 megabytes in size and data is retrieved on the bench-top at up to 300 kilobytes/second.

In conclusion, a commercial PIM program has been adapted to store scientific data in a readily accessible environment. Preliminary results show that such software can be very useful in real lab situations and assists the busy mass spectrometry professional to maintain control over the many different types of data encountered daily. The increasing memory sizes available for personal computers will do much to encourage and develop the application of such RAM-resident techniques.
COMPUTER-ASSISTED ANALYSIS OF MS/MS DATA FOR THE DIFFERENTIATION OF FOOD-BORNE MICROBIAL CONTAMINANTS

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The ability to differentiate among food-borne microbial contaminants is of great concern to the food industry. Current accepted methodologies often have difficulties distinguishing many pathogenic species from non-pathogenic species. In addition, these methodologies require up to 7 days for results and do not always provide an acceptable level of reliability [1]. Phospholipid analysis by tandem mass spectrometry has shown promise for rapidly differentiating several food-borne contaminants that are difficult to distinguish using current accepted methods [2]. This work presents an exploration of computer-assisted techniques in the interpretation of phospholipid MS/MS data for the differentiation of microbial contaminants.

When phospholipids, ionized by fast atom bombardment, undergo CID, they fragment at specific points that are common to all classes of phospholipids. Positive phospholipid ions fragment to lose their polar head group as a neutral loss, while negative ion daughter scans of the phospholipid ions detected in the neutral loss scans provide the fatty acyl information on the individual phospholipid species. The resulting phospholipid MS/MS data forms a 4-dimensional data space in which each phospholipid class is represented by a 3-dimensional map consisting of the phospholipid masses from the characteristic neutral loss scans along the first dimension, the fatty acyl masses from the daughter spectra along the second dimension, and the relative intensities along the third dimension. This data space is shown in Figure 1. The triple quadrupole mass spectrometer can be automated to perform this extensive phospholipid screening in a few minutes, on a single sample [4].

For the cluster analysis, twenty neutral loss scans were collected for each of the 5 possible phospholipid classes known to occur in the particular set of organisms studied. Data from three or four replicates were analyzed for each microorganism. Filtering of the data was necessary to reduce the number of dimensions (features) used in the analysis, and was accomplished on three levels: 1) a peak must occur in a minimum of 50% of the neutral loss scans collected for each phospholipid class 2) the scans thus filtered are then averaged 3) a peak must occur in 100% of the averaged spectra within each set of replicates to be used as a feature in the cluster analysis. The similarity index of two organisms (or sets, or groups) is given by the position of the vertical lines connecting the elements to be compared. The higher the similarity index, the more data features within the particular phospholipid class they share in common, and the more closely related are the spectra. An example of a dendrogram for the analysis based on phosphatidylglycerol is shown in Figure 2. Phosphatidylglycerol provides a high degree of discrimination for the two Salmonella species over all of the other organisms. In addition, E. coli, C. freundii, and L. monocytogenes were readily distinguished from each other. S. abortetubae and S. enteritidis were moderately separated.

For spectral matching, averaged spectra produced for the cluster analysis experiments, for each organism in a replicate set, were averaged to produce a library spectrum for that organism. A reverse-library fit routine was used for searching. "Unknown" spectra for matching against the library spectra were the filtered, averaged spectra from single organisms. The average of these matches, for the analysis of phosphatidic acid in all replicates, is shown in Table 1. The term Δ in Table 1, is the difference between the match value to itself and the next highest value. The higher the value for Δ, the more discriminating is the phospholipid class for that particular organism, as shown in Figure 3.

Both cluster analysis and spectral matching were useful for determining which classes of phospholipids were most discriminating among the different organisms, and both appear capable of discriminating among the organisms tested. Spectral matching is easier to implement and does not require pruning to reduce the number of features, but cluster analysis appears to be more discriminating and provides more information such as most similar samples and parent-offspring relationships. Neither of these techniques are able to incorporate all of the information contained in the phospholipid MS/MS data set shown in Figure 1.

1. Private communication with Dr. Edward Richter, Silliker Laboratories of Ohio, Inc.
The phospholipid data space obtained by FAB/MS/MS. The masses of the phospholipids in each class are obtained by neutral loss scans specific for each class. The daughter spectrum of each phospholipid species provides the fatty acyl information.

Figure 1.

Figure 2. Dendrogram for the analysis based on phosphatidylglycerol of several microorganisms.

Spectral Matching Discrimination

Figure 3. Degree of discrimination of different phospholipid classes for the organisms studied. The higher the $\Delta$ value, the more discriminating is the phospholipid class for that particular organism.

Table 1 The averages of the library matches for the analysis of phosphatidic acid in all replicates.
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ALGORITHMS FOR SEQUENCING PEPTIDES
FROM A VARIETY OF MASS SPECTRAL DATA

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SUMMARY

Algorithms for sequencing peptides have been written which can be applied to a variety of mass spectral data including high and low energy FAB MS/MS, high and low resolution FAB/MS and electrospray MS/MS. A unique feature of the algorithms is that all ions are considered to arise either as N- or C-terminus sequence ions or as non-sequence ions. Backtracking and feedback methods were used to enhance the speed of the algorithms. The numbers of amino acid residues in the generated sequences and the corresponding numbers of non-sequence ions were tracked for choosing the best scoring peptides.

ALGORITHM

A) DATA INPUT

Required input for the programs are: (i) observed spectrum: fragment ion masses and intensities, (ii) N-terminating group, (iii) C-terminating group, (iv) molecular weight of the peptide, (v) amino acid (aa) residue fragment ion tables, (vi) peptide scoring parameters, and, (vii) program control parameters. The aa residue fragment ion masses are divided into N- and C-terminal tables and each are expressed in two ways: (1) as aa residue fragment ion masses, and, (2) as mass changes upon fragmentation for each aa residue. The versatility of the programs originate from the fact that the aa residue fragment ion tables can be tailored to the type of data being analyzed and the list of amino acids considered. Customized tables were prepared for analyzing low and high energy FAB/MS and FAB/MS/MS data and for high resolution FAB/MS data. Also, these tables can be created to analyze data believed to originate from cleavage of all aa residues, of all individual and pairs of aa residues, of a selected list of any desired aa residues, etc. The computed peptides consistent with the input data were scored by peptide length, number of ion assignments per aa residue, number of unassigned ions, total ion current of assigned ions and by a weighted composite score.

B) SEQUENCING DETAILS

The sequencing can begin from either the N- or C-terminus and from either the high or low mass end, all being mathematically equivalent. For the following discussions, sequencing will be described from the N-terminus and from low to high mass.

1- Since fragment ions can arise from either the N- or C-terminus, all the experimentally observed fragment ion masses are mathematically recomputed as both N-terminus and C-terminus fragment ions where the C-terminus fragment ion masses are re-computed from the N-terminus side. Hence, all observed ions have two corresponding recomputed mass values, all expressed as N-terminus ions. All the possible recomputed ion masses, including the molecular weight of the peptide minus the mass of the C-terminating group, are sorted from lowest to highest mass after subtracting from each the mass of the N-terminating group. We refer to each of these masses as a "reconstructed ion mass (RIM)".

2- The lowest RIM mass value is searched in the appropriate N- or C-terminus aa residue fragment ion mass table for a match and the corresponding amino acid group is noted. All other fragment ions associated with this amino acid group are noted by searching the appropriate N- or C-terminating table of mass changes upon fragmentation and the matched masses are removed from the list of RIM values. This lowest RIM value is also considered to arise as a "bad ion", namely a non-sequence ion arising either from the peptide or from any other possible source.

3- Step 2 is repeated with the lowest mass value of the remaining RIM values until an amino acid sequence is found with a residue mass equal to the molecular weight of the peptide minus the N- and C-terminating groups, until the mass gap between the last assigned aa residue to a RIM value and the present RIM value is greater than the largest look-up mass in the aa residue fragment ion mass table, or the limits on the maximum number of aa residues or "bad ion" assignments has been exceeded. If a peptide sequence with the appropriate MW residue mass is determined, the sequence is stored as a possible answer if its weighted score exceeds the set lower limit.

4- BACKTRACKING- The last assigned aa residue or "bad ion" assignment is removed from the sequence list, the associated aa or "bad ion" RIM values are reincorporated into the list of unassigned RIM values, an alternate aa or "bad ion" assignment is considered and the sequence is extended as in Step 2. This backtracking procedure is continued until all possible combinations of assignments for aa's and "bad ions" are considered satisfying all the boundary conditions.
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**INPUT DATA**
1. Fragment ion masses
2. MW of peptide
3. Scoring and control parameters
4. C-terminating group
5. N-terminating group

**SPECTRAL RECONSTRUCTION**
1. Fragment ion masses recomputed as N- and C-terminus fragment ions
2. Re-express C-terminus fragment ions as N-terminus ions
3. Subtract N-terminating group mass from all ions

**FEEDBACK**
Partial sequences are now new N-terminating groups

**SEQUENCING**
Grow peptide from N-terminus/low to high mass using BACKTRACKING and LOOK-UP TABLES of aa residue fragment ions

**SOLUTIONS**
List of sequences satisfying boundary conditions

**C) POST-ANALYSIS**
For each peptide sequence solution, the unassigned fragment ions are analyzed for internal acyl fragment ions, internal immonium ions and for dehydration and loss of ammonia from the assigned ions. The scores for the peptide solutions are accordingly adjusted by the success of the post-analysis process. The list of sequence solutions are sorted, ordered from best to worst score and duplicates removed.

**D) FEEDBACK ANALYSIS**
Using the above described methodology, 40 fragment ions can be sequenced in about 20 minutes of CPU time. This time increases exponentially with increasing numbers of fragment ions because the backtrack method described above, but when feedback was incorporated into the algorithm the CPU time was dramatically reduced to less than 1 hour. The feedback process was incorporated in the following way. The above described backtrack method (Sections A-C) was implemented but instead of sequencing the whole peptide and all the fragment ions, only a partial peptide analysis is requested. In this way, for example, a peptide expected to have approximately 12 aa residues is sequenced to find all the first 5 aa residues consistent with the observed fragment ions. The mass of the N-terminating group plus the total mass of the aa residues for each partial solution is computed. These partial solutions are sorted and the duplicates removed. The remaining partial solutions were individually fed back into the backtrack routine (Sections A-C, above) with the only major change being that the masses of the partial solutions are now the N-terminating masses and the backtrack method proceeds to sequence all the RIM values greater than the mass of the N-terminating feedback mass.

**CONCLUSION**
The procedures described above were found to rapidly sequence peptides from a variety of low and high energy FAB mass spectral data. In all cases studied to date, the correct answer was always one of the top ten candidates. For multiply charged molecular ions studied by ES/MS/MS, the input spectral data were the observed ion masses and all possible multiple masses corresponding to all possible charge states. The score for the correct answer was very low because degenerate ions were used to generate the peptide sequences. Upon removing answers derived from degenerate ions, the ranking for the correct answer was very high. This exercise demonstrates that a more efficient procedure for sequencing multiply charged ES/MS/MS data may be to individually analyze each possible combination of charged species and then ranking all the answers.
Fast atom bombardment has become an important ionization technique in the analysis of biomolecules such as peptides and nucleotides and has allowed the determination of their primary structure. Reports have been published showing that the amino acid composition and sequence of unknown peptides can be determined from their FAB spectra. Computer algorithms have been developed to assist in the interpretation of peptide spectra which can be complex under the conditions used in FAB/LSIMS analyses. Although there are different approaches to the computerized or manual interpretation of FAB/LSIMS spectra, they all share a common difficulty which is the extraction of meaningful information from the spectrum. This is due to the fact that the spectra generated are heavily contaminated by background peaks which are produced by radiation damage of the matrix and the analyte by the high energy bombarding ion/atom beam. A FAB/LSIMS mass spectrum represents a quasi-continuum of peaks as illustrated in Figure 1a. The octapeptide, WRVFVHPF, analyzed in a glycerol matrix gives background peaks at every mass. The background intensities are distributed as a function of mass (decreasing with increasing mass) which makes it difficult to identify peaks that are related to the analyte. This “peak-at-every-mass” syndrome can be detrimental to computer algorithms that predict the mass of sequence ions and rely on the presence of specific ions in the spectrum for the determination of the probable sequences for the peptide.

In order to minimize the number of possible sequences in interpreting the spectrum while maintaining the structurally important information, preliminary treatment of the experimental spectrum is indispensable. The efficiency of any algorithm used for spectral interpretation is dependent on the filtering method used to remove the background from the spectrum. There are basically two approaches that can be used for background removal: I) direct subtraction of baseline using a threshold value, and II) use of an empirical method for background calculation and removal. Direct subtraction of background from a FAB/LSIMS spectrum is very difficult. This is due to the fact that the intensities of the background peaks vary with the mass. Furthermore, because the background peaks depend on the nature of the matrix and the analyte due to strong analyte-matrix interaction a simple subtraction is not practical. The use of an empirical method such as the one suggested by Ishikawa (1), although more efficient than direct subtraction, suffers from serious constraints related to the high number of residual peaks.

In seeking a background treatment method that would be objective, automatic, and concentrate useful information from FAB/LSIMS spectra, we have developed a compound-independent algorithm that removes background, without removing peaks of weak intensities due to the analyte, by calculating the frequency of peak intensities. The algorithm that is described here is based on the statistical assumption that the background signal is the one with the highest frequency in the mass spectrum. The algorithm developed is based on the following hypotheses: I) the background intensities are distributed as a function of mass, II) in a mass interval, the maximal number of peaks in a range of intensities (I± Al), determines the intensity of background. There are three major components to this algorithm. Section A controls the input data as a mass/Intensity list and calculates the number of intervals as a function of interval length defined by the user. Section B is an iterative procedure which evaluates and filters the background intensity for each interval according to hypothesis II. Finally, section C allows for the isotopic ratio correction if requested by the user. Isotopic peak correction may be made in order to minimize the total number of peaks remaining after the filtering procedure. The program “STRIP” was written in ANSI Fortran 77 and runs on the VAX-Station 2000 and 3100 (VMS 5.0 or later). The source program is transportable without modification to any computer having a Fortran compiler. The CPU time required for filtering a spectrum containing around 1500 mass peaks is approximately 1.5 seconds.

The algorithm has been applied to FAB/LSIMS spectra and an example of the results obtained is shown in Figure 1. The figure shows the LSIMS spectrum of the octapeptide WRVFVHPF (1a), the background that has been removed (1b) and the residual peaks (1c). It can be seen that most of the background peaks have been eliminated and that the useful spectral information has been substantially concentrated. The sequence ions present in the spectrum have been retained in the corrected spectrum (1c). Since the algorithm conducts a statistical treatment of the background and removes the peaks according to their intensity frequency, it is matrix independent and therefore can treat any type of matrix. This has been verified by comparison of the raw and treated spectra of bradykinin analyzed in glycerol and thioglycerol. These spectra have been found to be very similar in appearance indicating the universality of the approach. Interestingly, the treated spectrum obtained in glycerol showed the presence of more intense sequence ions.
It is also essential when using methods for background treatment that the algorithm removes most of the background signal while retaining small peaks that belong to the analyte. This aspect is especially important in the analysis of compounds like peptides where several sequence ions are of weak intensities. In order to demonstrate the S/N resolution of the new algorithm, Figure 2 shows an expanded portion of a raw FAB/LSIMS spectrum and the corresponding region after spectral treatment. It can easily be observed that the algorithm has excellent S/N resolution and that it can retain peaks that are only slightly above background signal, thus reducing the possibility of removing analyte peaks which are relatively weak.

In order to evaluate the overall efficiency of the new algorithm, the results obtained by this method and that of Ishikawa were compared for several spectra of peptides. Table 1 gives the number of total peaks in the spectra, the number of sequence ions observed before and after treatment and the percentage of spectral information enhancement. It can be observed that our method rejects on the average 90% of the peaks in the initial spectra compared to only 15% for the method of Ishikawa and that both approaches retain over 90% of the sequence ions. Therefore, the algorithm enhances the information by 1000% as compared to 25% for the method of Ishikawa which represents a significant improvement in the quality of information. Thus, the use of such a general background treatment and removal method can be extremely useful in the computerized analysis and characterization of biomolecules by desorption ionization techniques and also for other general applications requiring the elimination of similar background interferences.

REFERENCES


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<th>Raw data</th>
<th>STRIP algorithm</th>
<th>ISHIKAWA's algorithm</th>
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</thead>
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<td>Number of peaks before filtering</td>
<td>Number of observed sequence fragments</td>
<td>Percentage of observed sequence fragments</td>
</tr>
<tr>
<td>963</td>
<td>28</td>
<td>2.82</td>
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<tr>
<td>967</td>
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Organic sulfonium salts exist as pre-charged species in the matrices normally used for fast atom bombardment mass spectrometry. Positive ion FAB mass spectra of the sulfonium salts exhibit the intact cation as the dominant species. Generally, the fragment ions produced in the spectra are of 30-40% relative abundance. Although other onium salts commonly exhibit rearrangement ions in their FAB mass spectra, the fragment ions produced from sulfonium salts are all products of simple cleavages. Cluster ions CAC* and CACAC* were also observed to occur in the spectra when higher concentrations of the sample in matrix were used. These cluster ions provided confirmation of the salt's molecular weight when beam-induced reduction reactions were suspected to be complicating the spectra. A single selenonium salt was also included in this study. The FAB mass spectra of a triphenylselenonium hexafluoroarsenate salt contained only the intact cation, and no fragmentation was observed (Figure 1). This the first report of a FAB mass spectrum of a selenonium salt.

Semiempirical calculations of the sulfonium salts were performed to determine gas phase stereostructures. MOPAC 5.0 calculations were used to determine the minimum energy structures of the cations. The bond lengths between the sulfur (with a localized charge) and any of the three groups bound to it are in the range of 1.73 to 1.80 Angstroms. These bond lengths are somewhat shorter than sulfur-carbon bond lengths typically found in neutral compounds. Though MOPAC calculations used as such can not predict the preferential cleavage of a substituent, in all of the eighteen different sulfonium salts calculated, the weaker bonds (longer) were cleaved more readily in their FAB mass spectra. Additionally, the MOPAC calculations show that the geometrical orientation of substituents depends directly on their identity. For example, the benzylidimethyl sulfonium salt exhibits a methyl-S-methyl angle of 143 degrees, whereas the methyl-S-benzyl angle is only 108 degrees. For aromatic sulfonium salts, the angles of the three substituents around the sulfur are all approximately equal, and the substituents are held far away from each other.

Positive ion FAB mass spectra were recorded using meta-nitrobenzyl alcohol as the matrix. This was found to be far superior over the more common glycerol matrix. The spectra indicate that cleavages of the sulfur to carbon bond were most common. Losses were typically associated with one or two phenyl groups for the aryl sulfonium salts, and alkyl groups for salts with these groups. Using higher concentrations of the salts in the matrix produces the cluster ions CAC* or CACAC*.

An intriguing example of the sulfonium salt FAB spectra is given in Figure 2. It is a doubly charged salt with a dication observed at m/z 278. The mass at m/z 701 is the cluster ion formed by two cations and one anion. Loss of a phenyl group and an anion from the cluster ion forms the fragment at m/z 479. A minimized stereostructure of the doubly charged salt as calculated by MOPAC is given in Figure 3.

The sulfonium salts presented here all provide excellent quality mass spectra. For singly charged salts, the intact cation is the dominant species in the spectra. Fragmentation of the salts is centered about simple cleavages of the sulfur-carbon bonds. Semiempirical calculations confirm the preferential breakage of weaker (longer) bonds which form the fragment ions observed in the spectra.
Figure 1

Figure 2

Figure 3
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DEVELOPMENT OF ABSTRACT FACTOR ANALYSIS TOOLS IN A WORKSTATION ENVIRONMENT: THE ROLE OF SUBSTITUENT PROTON TRANSFER IN THE ORTHO EFFECT

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Harrison has published a series of papers on the fragmentation pathways of benzoic acids and related compounds. In these studies, he mentions that proton donation by the o-amine and o-hydroxy substituents clearly account for exaggerated dehydration/decarboxylation ratios of those compounds. Curiously, the o-ido compound shows a similarly significant dehydration/decarboxylation ratio. Weisz, Andrzejewski and Mandelbaum demonstrated the significant involvement of substituents in the dehydration of 2-haloresorcinols under electron impact conditions. This suggests that the exceptionally high dehydration/decarboxylation of the o-ido benzoic acid may be mediated by proton donation from the iodo substituent. We are developing tools to predict these fragmentation patterns. In general, Hammett substituent effect correlations would be expected to predict these ratios.

Application of the Hammett substituent effect formalism has long been used in rational molecule design, quantitative structural activity relationship studies (QSAR) and explanation of mass spectrometry fragmentation patterns. The Hammett formalism has steadily evolved from the original correlations between pH values of substituted benzoic acids and target reaction rates. Saponification rates of methyl benzoates, and hydrolysis rates of benzyl chlorides were also used to correlate experimental values. Later studies separated the meta and para substituent values into different families of sigma values. Many studies have investigated the linear relationship between various sigma values and mass-mass spectrometry peak intensity ratios, NMR shift values, photo-ionization energies and other laboratory quantities. Experience with the Hammett formalism revealed that there were actually independent, additive effects. Saturated and aromatic molecules typically revealed these differences more clearly, and the strongest two effects are called field and resonance effects. Taft and others realized that a better form of the Hammett equation required inclusion of these effects in the equation.

$\ln \left( \frac{k}{k_0} \right) = C_F \cdot F + C_R \cdot R$

Swain and Lupton published a seminal work gathering the hundreds of lab values used for the various families of sigma values. By treating these with statistical methods, they defined field and resonance values, F and R, which represented the body of Hammett substituent constants. Linear transformations of this form are more easily recognized when they are represented as a rotation of a two dimensional object, such as a polygon made of the body of data points. Cursory analysis of the literature values for dehydration/decarboxylation values of substituted benzoic acids under various CIMS conditions suggested that there was indeed an underlying two dimensional linear transformation, where the classical one dimensional transformation is not present. We have developed abstract factor analysis tools to correlate substituent effects in mass spectral data.

Factor analysis is the tool of choice to determine how many dimensions are in fact necessary to represent a chemical problem to the limit of experimental error. The simplest form, abstract factor analysis, yields a set of numbers which describe the system, but which in themselves do not necessarily offer chemical insight. Target factor analysis is the more subjective area where actual chemical measurements, such as photoelectron spectral data, $^{13}C$MR shifts, proton affinities, Swain and Lupton F & R values or other measurements are identified as predicting the current laboratory data. Additional uses of factor analysis in chemistry include the identification of the number and spectra of absorbing species in UV-VIS spectroscopy, and identification and isolation of mass spectra from incompletely resolved gas chromatography - mass spectrometry chromatograms.

Recognizing the pivotal role of factor analysis to perform tasks in the mass spectrometry lab ranging
from the routine manipulation of mass spectrometry chromatogram data to the fundamental investigation of substituent effects and their mechanistic implications, we have developed computer software that runs in a highly interactive, mouse based graphically intensive environment. The library supporting this effort was modularly designed, and a portion is separately available as Real number and Matrix arithmetic applications. These applications incorporate correct management of significant digits, incorporation of comments and a prose text appendix to matrix data files. The overall emphasis in this library is ease of preparation, interpretation, and use of data sets by people. The initial use of factor analysis in our lab is the investigation of the ortho effect, beginning with the well studied hydrogen and methane chemical ionization mass spectra of ring substituted benzoic acids.

The central software is now finished, and benchmark studies are being performed on various literature data sets to gain experience. Surprisingly, this benchmark data is not from mass spectrometry. Carbon-13 NMR shifts are well studied, and to the first approximation the changes in the shifts upon substitution are additive. The chemical shift value appears to correlate with the Hammett substituent constants. Theoretical work, from second order perturbation theory, predicts that the chemical shift will correlate with the electron density at the nucleus. The shifts of the substituted carbon appear to vary with solution pH in a pattern consistent to the dehydration/decarboxylation ratio changes under chemical ionization mass spec. The NMR shifts for a family of substituted benzenes, including benzoic acids, was examined with the factor analysis software. This analysis gave good results. Although the literature benzoic acid data was sparse, and used hydrazine adducts and ammonium salts, shift values were easily assigned based on these predictions. The table below shows these rough predictions, and our assigned spectra. After the ambiguous assignments were resolved, the difference between lab values and the predicted values was typically within a ppm.

We have developed powerful, deeply interactive, environment to evaluate various linear models explaining substituent effects in mass spectrometry. This environment is a springboard toward applications as diverse as evaluating the origins of the ortho effect and developing spectral purification software.

### Table: Predicted Spectra and Initial Shift Assignments

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<th>ID</th>
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<th>c-phenot</th>
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<td>131.2</td>
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<tr>
<td>s(k)</td>
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<td>150.7</td>
<td>150.7</td>
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</tr>
</tbody>
</table>

Initial Predictions:

The benzene values were supplied in the original data sets, the others were fixed. The ortho compound was the hydrazine adduct. The benzene acid value is from Irk Jankowski. This is used as an initial guide to assign the shifts to data spectra.
Tandem mass spectrometry (MS/MS) has proven to be a powerful analytical tool for both complex mixture analysis and structure elucidation of unknown compounds.

We applied a newly designed instrument control language (ICL™) to the automation of a GC/MS/MS system of BEQ geometry (Fig. 1). Hybrid MS/MS instruments (BEQ, EBQ or EBEQ) combine the versatility of double focusing magnetic sector instruments (e.g. high resolution, high mass) with the simplicity of quadrupole instruments (ease of operation). However, since these two parts are based on significantly different physical principles, hybrid instruments require precise tuning and sophisticated coordination of their operations for optimum performance. Now, the new completely automated design of the BEQ makes this combination of the high resolution magnetic sector and the quadrupole mass analyzer far more routine than previous systems.

The ion source tuning and the setup of all control voltages within the quadrupole section (Fig. 1) are automatically optimized and implemented by routines implemented within the user interface (Fig. 2). Figure 3 displays an ICL routine written to automatically acquire the daughter ion MS/MS spectra of five different precursor ions. The chromatogram of this GC/MS/MS experiment is shown in Fig. 4. Other subroutines such as RETTIME and RETINI allow the automated precursor ion selection to be extended to quite complex samples with many selected precursors. The maximum switch-over-time between two precursors is less than 1 second. This allows the analyst to prepare mass menus with as many as 32 different precursor ions per time window, with each time window being less than 1 minute. Typical mass values monitored could range from 20 to 500 daltons. In addition, the flexible interface offers the user the option of selecting different MS/MS experiments within a given chromatographic run.

Thus, the application of ICL to the operation of a hybrid mass spectrometer of BEQ design combines the highest degree of analytical flexibility with simplicity and reproducibility of instrument operation.
Fig. 2: User interface controlling all MS/MS related experiments (daughter ion scans, parent ion scans, neutral loss scans, etc.).

Fig. 3: ICL procedure customized for monitoring the different precursor ions of peaks in the chromatogram in Fig. 4.

Fig. 4: Reconstructed ion chromatogram (RIC) of a GC/MS/MS run with five different automatically selected precursor ions.
INTRODUCTION

Quantitation of mass spectral data presents a wide range of computational requirements, including the proper handling of a large variety of data types, targetting protocols, calibration methods, report formats and even terminologies.

This paper describes a computer program, OpusQuan, which embraces all known types of quantitation calculations and which is conveniently driven by means of user defined forms for all input and output requirements.

Although designed to operate with a commercial data system, it has now been extended to accept input in the form of a generic ASCII peak list containing the minimum information of mass, retention time, and intensity.

GENERAL

Figure 1) shows the general relationship of OpusQuan to its processing environment.

- When used in conjunction with the commercial OPUS system, OpusQuan performs all the operations of chromatogram generation, peak detection and Integration, targetting and quantitative calculations. These operations are performed interactively, or in background (batch processing) mode with the Opus File Services (OFS) utility.

- When used independently to process ASCII peak lists, the parameter set is much smaller, as no peak detection is to be performed, but with no reduction in the targetting or calculation flexibility.

CONSIDERATIONS

A major step in the generalization of a quantitation package is to reduce to a manageable number the terms used to describe the various components in a quantitation mixture. Typically, reference is made to one, or more, of the following: Unknown; Analyte; Internal Standard; External Standard; Surrogate Standard; Recovery Standard; Standard Standard; Clean-up Standard; Retention Standard; etc.

OpusQuan 'knows' of only 3 component types, namely: Analyte; Standard; 'Super' Standard, but will apply the appropriate calculations to provide the conventionally accepted responses to all of the above. This is achieved by means of a self evident context within a table and the value of 2 parameters associated with each component. The hierarchy for Calibration and Quantitation calculations is depicted in Figure 2).
Historically, the calculations for any particular analyte have been defined by the type of standard(s) to which it is referenced, producing a seemingly large number of calibration and quantitation methods.

We believe that in essence there are in fact only 4 such methods, as illustrated in Figure 2).

Each analyte is referenced to one or more standards; these standards are not given special names. Instead, the method to be used is defined by 2 yes/no keywords.

Thus, for example, when calculating the recovery of surrogate standards, the surrogate standard becomes an 'analyte' and its internal standard becomes a 'standard'.

TARGETTING CRITERIA

OpusQuan locates targetted components on the basis of:

- A primary ion mass
- A Retention Time 'window'
- The relative abundance AND retention time of any number of additional ions

There are parameters for flagging, but still using, the response of components which may fall either the Relative Abundance or Retention Time alignment criteria.

The response of a component may optionally be defined to be the sum of the responses of all targetted ions, or simply the response of the Primary ion.

TOTALS

The total response of a homologous series may be reported, together with a 'maximum possible' amount, for any homolog falling the relative abundance criteria. This facility is particularly applicable to simple applications.

FORMS

All parameters and results for each analyte are stored in a database. The forms facility allows any item from this database to be displayed, and reported in a user specified position and format.

For example, one form may be designed to display selected parameters from the database on the basis of 1 row per analyte, whilst an alternative form could display all the parameters for an analyte on a 1 page per analyte basis.

CALIBRATION METHODS

The calibration methods available are:

- Curve Types: Mean; Linear; Exponential; Logarithmic; Quadratic; Inverse Quadratic; Piece-wise Linear.
- Origin Treatment: Force; Include; Ignore
Mass spectral libraries represent major time and resource investments for most laboratories. To change instruments usually means starting all over again, especially if these are made by different manufacturers. A new JCAMP-DX mass spectral data exchange file format is being developed in cooperation between Finnigan, VG, Hewlett-Packard and other manufacturers. It is capable of data exchange between different computer platforms. Unlike an older EPA format, the new JCAMP-DX is capable of exchanging a wide variety of data, including high resolution and high molecular weight mass spectra. It is also in an ASCII format, but file structure rules are not as strict as with the older EPA standard. All the necessary information for mass spectral libraries transfer is available in the new format and is therefore suitable for a library transport. We have recently acquired VG Analytical Autospec Q and succeeded in transferring the specialized toxicological (TX) and general purpose (GP) mass spectral libraries from Finnigan INCOS format to the VG OPUS format. The Finnigan INCOS library editor program (EDLB.MS) data were sent to a computer terminal screen through a short procedure. An IBM PC compatible computer was used as a terminal and was running a terminal emulation program (eg. Smarterm 240 or EM4010). The data were captured by the terminal emulation program into an ASCII file on a PC hard disk. An application program was written in Quick BASIC (GCMS.EXE, released to public domain) and was utilized to convert the data to a JCAMP-DX format in a new file. This program is also capable of reading/writing EPA and Finnigan ITDS data files and creating HPGL files for plotting on HP and compatible plotters or export to desktop publishing programs. JCAMP-DX files were sent from an IBM PC compatible microcomputer using the same terminal emulation program, utilizing the Kermit file transfer protocol. IBM PC compatible was connected through a RS-232C serial port to the DEC VAX station 3100 running VMS and VG OPUS software. The OPUS SPECBASE program is capable of reading in the JCAMP-DX ASCII file and converting the data to searchable libraries. (Both the GCMS.EXE program and mass spectral libraries are available from the author.)
Chemstation macros have been written for the transfer of S.I.M. or T.I.C. data from the Pascal 3.2 or UNIX-based MSD chemstations to a centralized Vax minicomputer. These macros are run during data acquisition, or postanalysis in a batch mode. The macros begin by sending the text string "START_OF_FILE XYZ" out the serial port of the chemstation (where XYZ are the user's initials). The time/abundance data for a given ion is then sent out the serial port, followed by the text string "END_OF_FILE".

On the Vax, BASIC programs are running continuously to read data being sent from any MSD chemstation connected via a serial port. Upon receipt of the string "START_OF_FILE" the BASIC program opens a file and stores the subsequent time/abundance data for a given ion to an ASCII file. The file is closed upon receipt of the string "END_OF_FILE". The time/abundance data in this initial file is then interpolated from the Hewlett Packard variable time intervals to a constant time interval format, required for the P.E. Nelson ACCESS*CHROM data files. Finally, the interpolated ASCII file is converted to the binary .RAW file type used in ACCESS*CHROM. This final file is stored in XYZ user's Vax directory. The original time and date of analysis are transferred with the raw data and are found in the sample name field of the final .RAW file.

Files created on the Vax by this procedure are sequentially numbered with the following format: XYZ_DATE_001.RAW. A list of files successfully transferred by this procedure is stored as an ASCII .LOG file in the user's directory. The final three digits of the Vax file are reset to 001, and a new .LOG file created, whenever two hours elapse between file transfers or if the initials of the user change.

**FLOWCHART OF MSD -> VAX DATA TRANSFER AND INTERPOLATION**

MSD

- MSD S.I.M. OR T.I.C. TIME/ABUNDANCE OUTPUT

VAX

- PROGRAM ON THE VAX READS AND STORES MSD DATA INTO THE ASCII FILE "XYZ_DATE_001.MSD".

- CONVERSION PROGRAM ON THE VAX CONVERTS AND INTERPOLATES "XYZ_DATE_001.MSD" FILE TO A "XYZ_DATE_001.RAX" ASCII FILE.

- COMMAND FILE ON THE VAX CONVERTS THE "XYZ_DATE_001 RAX" ASCII FILE INTO THE BINARY "XYZ_DATE_001.RAW" FILE. THE CONVERSION IS "LOGGED" AND THE TWO ASCII FILES DELETED.
DATA TRANSFER FROM A DEC PDP-11 BASED MS DATA STATION TO A MS-DOS PERSONAL COMPUTER.

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The modern instrumentation for mass spectrometry is getting better and better, both considering the measurement hardware and the data stations. The software for data acquisition and reduction, as well as for the management of all the instrumental parameters allows nowadays a very easy operation of the machines. On the contrary, very little effort has been devoted to give the possibility to get good quality graphic presentation of data. Most MS data systems are equipped indeed with low density 9 pin matrix printers, and the software, usually, does not even provide the correct drivers for high quality laser printers or pen plotters.

We report here about the preparation of good quality graphic outputs from a NERMAG R3010 mass spectrometer equipped with a DEC PDP-11/73 based data station, using "KERMIT" to transfer files to an IBM compatible computer (Figure 1).

As the software of this machine (SIDAR) is not able to generate ASCII tables for chromatograms and spectra, we recovered them from the printer dump files generated on the disk when the printer is set off line, as these files can be handled as normal files. KERMIT can transfer the ASCII tables obtained to a personal computer. KERMIT can transfer binary files as well, but, of course, this can be useful only if a suitable software for their elaboration is available on the PC. The ASCII table can be then imported and elaborated using one of the powerful tools available for MS-DOS computers. In particular, the graphic output can be generated by the use of a simple spreadsheet (we used Borland Quattro Pro 1.0) or a presentation graphic program (we used Harvard Graphics 2.12). The main advantage of these programs is the possibility to customize
the format of the plot, also by text and graphic annotation. Moreover the final result can be sent to plotters or laser printers or exported to word processors such as Microsoft Word 5.

The method has been tested also on a VG 70-70E equipped with a DEC PDP-11/23 based data station. The software in this case is able to generate directly ASCII tables, but only for spectra, whereas it was not possible to handle chromatograms.

In Figures 2 and 3 are reported two examples of customized plots of one chromatogram and one spectrum, respectively.

![Figure 2](image)

**Figure 2** T.I.C. chromatogram relative to a CZE-ISMS experiment of a mixture of quaternary ammonium salts. The plot of the chromatogram was obtained using a spreadsheet, as the structures were made using a molecule drawing program.

![Figure 3](image)

**Figure 3** Ionspray mass spectrum of a sample of 6-hydroxymellein. The plot of the spectrum was obtained using a spreadsheet, as the structure has been drawn by a molecule drawing program.
USE OF A NETWORK-BASED LIMS SYSTEM TO AID IN
QUALITY ASSURANCE OF ENVIRONMENTAL GC/MS DATA

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William L. Budde, U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

The E.P.A. is responsible for analyzing, evaluating, and interpreting enormous quantities of environmental monitoring data, much of which is quantitative GC/MS analyses of hazardous substances. Samples are collected in the field and analysed for a known list of "target" compounds. This analysis procedure involves multi-level calibrations, use of surrogate and internal standard compounds, and a substantial level of additional QC. The goal of this procedure is to obtain a quantitative estimate of the amount of each "target" compound present in the original sample.

The data analysis process has been automated previously. GC/MS software is available to operate directly on laboratory instrument computers or attached PC's which processes data obtained by the instrumentation software. We have recently installed a PC-based Laboratory Information Management System (LIMS) to aid in further data evaluation and interpretation. The laboratory software performs all quantitation calculations, converts all results to the proper units, and performs all "bench-level" QC to aid the analyst in assessing data quality. The end result of this software is the creation of a data file according to the EPA Standard Format for Data Transmission of Laboratory Results (Dec. 1987) which can be transferred to a LIMS system or other central data repository. This file includes all final results together with sample documentation, intermediate values, and an assortment of flags and check fields necessary to prevent and/or detect unauthorized manipulation of data. This system allows the LIMS to replace most uses of instrument-generated hard-copy output.

The LIMS system is currently operational on our building-wide Local Area Network (LAN) so access is available to all laboratory personnel. This LAN connects most projects located throughout EPA's Cincinnati facility and is constantly being expanded. Currently there are nine separate network rings connected by a building-wide "backbone". There are also connections to other nearby EPA facilities in the Cincinnati area as well as provisions for accessing central EPA computers in Washington and Research Triangle Park, NC. This provides access to all necessary personnel from log-in clerks and GC/MS operators to lab managers and quality assurance officers. Clients could also be tied in to the system directly if desired.

The LIMS system provides full tracking of all samples analyzed from receipt to final dissemination of results. Samples are logged in by the clerk as they are received and assigned an order number which is used to track the sample throughout the laboratory. Tests are assigned and service groups identified. This allows the laboratory manager to have immediate access to the status of all samples currently being processed. Schedules are determined for each instrument based on numbers of samples and required due dates.

All instrumentation is being interfaced directly to the LIMS via the LAN so data can be transferred directly from the instrument data system to the LIMS. Prior to loading any data into the LIMS, it is essential that data be fully reviewed by the analyst. The above-mentioned software has been installed on all GC/MS systems to assist the analyst in this review and QC verification. Required laboratory QC might include duplicate analyses, use of matrix spiking solutions, addition of surrogate compounds to each sample, and periodic analyses of blanks, calibrations, and tuning compounds. Checks may also be made on peak areas and retention times of key compounds. In addition, all results must be compared to the valid calibration range and sample-wide parameters such as the elapsed time between sampling and analysis must be checked.
This software has been used in other laboratories for five years. It has been designed in a generic "method-independent" manner and will interface to most if not all commercially available GC/MS systems as well as some GC/EC systems. At the conclusion of this process the software transmits all results to the LIMS in the above mentioned EPA Standard Format. This avoids hand-transcription of results thus streamlining operations and preventing errors from occurring. We have also purchased software for inorganics instrumentation (AA, ICP, etc.), but this has not yet been tested. This software, like the GC/MS software, will automatically collect data from instruments, provide reports and QC checks, and produce a file in the EPA Standard Format.

A program has been written to transfer any instrument results presented in this Standard Format to our LIMS. It operates by reading the Standard Format File, parsing each line into a temporary data base, and then transferring the necessary fields into the appropriate places within the LIMS. All required completion flags must be set within the LIMS to indicate that the results have been entered. The initial version of this program is operational, but modifications are needed in order to capture all QC data and direct it to the appropriate place within the LIMS.

Programs are also available within the LIMS system to allow managers to review data and evaluate results. A wide variety of reports, each of which can be customized easily, can be generated. We are currently working on client reports for final results for one of the tests. Ultimately, all client reports will be generated directly on the LIMS.

Other functions within the LIMS maintain audit trails of all critical operations and make long-term assessments of laboratory QC. While the analyst will always perform all required bench-level QC, the LIMS will track long-term performance. This is useful for establishing control limits on key instrument parameters as well as monitoring laboratory performance and generating instrument control charts. The LIMS could also schedule instrument maintenance by using the above data. In addition, clients could be given direct access to the LIMS data so they could verify field QC automatically. As an example, two samples submitted as unrelated "unknowns" to the laboratory might in fact be aliquots of the same sample (field duplicates). The client could use the LIMS to calculate percent differences for each measured concentration thus verifying laboratory reproducibility. Other client uses might include the preparation of site evaluation reports and/or maps.

The LIMS also has administrative roles. It can provide management and clients with sample tracking reports as well as manage laboratory finances. Invoices can be prepared automatically and cost estimates generated for new work. Each test can be assigned a cost, and each client given a discount rate, so totals can be automatically computed.

Much work remains to be done. We are currently logging in all samples as received, and the GC/MS laboratories are almost ready to transfer all results to the LIMS using the above-mentioned software. We have just begun to define the QC requirements and set up data base structures to fulfill these needs. Other instrumentation, such as inorganic and biological analyses have yet to be interfaced.

The ultimate goal of this work is to provide a paper-free method of operation, where results can be evaluated quickly and accurately, and then transferred directly to clients in a timely manner. It is hoped that by use of the EPA Standard Format for Data Transmission, all EPA monitoring programs can be linked together with data being shared between them. State and Local agencies should also participate. This system is intended to serve as a prototype of how the above goals might be accomplished. We have already worked with the State of Ohio and hope to expand these contacts considerably in the future.
Sample Matrix Subtraction in Full-Scan Gas Chromatography/Mass Spectrometry

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One function of our gas chromatography/mass spectrometry (GC/MS) laboratory is to identify analytes which have been detected by gas chromatographic systems. These GC systems usually use electron capture (EC) detectors, and the sensitivities of these detectors are obviously different than those of the mass spectrometer: non-electron-capturing species are not seen by the EC, but are seen by the mass spectrometer, and the response of electron-capturing species is proportionately greater with the EC than with the GC/MS. In addition, EC-GC conditions are frequently different from those of GC/MS, involving dissimilar columns and conditions. The result of all this is that it is difficult to correlate EC-GC peaks with GC/MS peaks.

Figure 1a shows a full-scan GC/MS chromatogram of a concentrated milk extract. The EC trace (not shown) of the same extract shows almost a flat baseline, with a few very small peaks. The GC/MS sees many fatty acids, which are not seen by the EC. The mass spectrometrist can waste time on these "matrix" peaks, which have nothing to do with the analytes he or she is asked to confirm or identify.

An important feature of the milk extracts is that the matrix peaks are part of the nature of the extract; they almost always show up on the GC/MS trace, though in varying proportions. It dawned on us that we could get rid of matrix peaks by subtracting their spectra from the corresponding spectra of a sample.

A computer program was written to subtract matrix peaks from a sample extract total-ion-current (TIC) chromatogram. The program subtracts spectral intensities of a blank matrix extract from those of a sample extract. Refer to Figures 1, 2, and 3. Figure 1a shows a TIC chromatogram of a milk extract containing six pesticides. Figure 2a shows a TIC chromatogram of a milk blank extract (no pesticides). Figures 1b and 2b are spectra of scan 415 of the TIC chromatograms shown in Figures 1a and 2a, respectively. Figure 3b is the spectrum of Figure 1b minus that of Figure 2b. The subtracted spectra are joined to create a new TIC chromatogram, shown in Figure 3a; note that only the pesticide peaks remain.

Spectral subtraction proceeds as follows. For each scan, the intensities of the matrix spectrum are subtracted from those of the sample spectrum. If the net is less than zero, it is set to zero. Figures 4a-4c compare the performance of matrix subtraction to that of background subtraction (part of the mass spectral data system). Figure 4a is the sum of spectra 841-846 (which is that of p,p'-DDE) from the TIC chromatogram shown in Figure 1a. Figure 4b is the spectrum obtained using background (i.e., on one side of the peak) subtraction. Figure 4c shows the sum of spectra 841-846 of the matrix-subtracted TIC chromatogram (Figure 3a).

It is important that the blank (matrix) concentration be greater than that of the sample (to make sure that the matrix intensities are larger than corresponding intensities of the sample, so that the net intensities will be set to zero). Figure 5a shows a matrix-subtracted TIC chromatogram using a matrix concentration equal to the sample concentration. Figure 5b shows a matrix-subtracted TIC chromatogram using a matrix concentration 1.5 times that of the sample concentration. Figure 5c shows a matrix-subtracted TIC chromatogram using a matrix concentration 3 times that of the sample concentration.

The computer program runs on an IBM-compatible PC, and uses Nermag’s Spectral 30 mass spectrometer data file format specifically.
Gelatin Films as Standards for Quantitative Molecular SIMS Analysis of Biological Tissue

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Analysis of physiologically important molecules in biological tissue by secondary ion mass spectrometry (SIMS) has become an area of much interest in recent years. In any quantitative SIMS experiment, the presence of matrix effects requires the use of standards that have a matrix composition similar to that of the unknown. In the case of biological tissues, such standards are not generally available. One potential approach, often used for elemental analysis of biological tissue, is to use doped gelatin or Epon plastic as standards (1,2,3). Gelatin has several advantages; its elemental composition is similar to that of tissue, it is compatible with the high vacuum of the SIMS instrument, and it is easily doped with a variety of molecular species. This preliminary report addresses the potential use of gelatin films doped with molecular species as standards for organic SIMS of biological tissues. In particular, linearity and detection limits are explored for several different compound/gelatin standards. Finally, the feasibility of using gelatin films as standards for molecular imaging SIMS is discussed.

Two percent solutions of porcine gelatin A (Sigma) were prepared in distilled water at ~80°C. Stock solutions of analyte compounds were prepared and serial dilutions used to generate standards of various concentrations. Aliquots of the standard solutions were then added to the gelatin. Concentrations were calculated on the basis of dry weight for both the gelatin and analyte compounds. Compounds used in this study include acetylcholine, cocaine, epinephrine, methylene blue, tetrabutyl ammonium bromide and tetrapentyl ammonium bromide. Spin-cast doped gelatin film samples were prepared by depositing 1 drop of the gelatin/compound mixture (while still warm and not too viscous) onto a 1 cm$^2$ silicon wafer which was spinning at 12,000-15,000 rpm on a May Spinning Disk Aerosol Generator. Spin-casting of the gelatin solutions results in a homogeneous film which completely covers the substrate silicon. Film thicknesses, as measured with a surface profilometer, were found to be 30-50 nm. This was sufficiently thin to eliminate the need for charge compensation during SIMS analysis. All analyses were conducted in depth profile mode on a Cameca IMS-3F ion microscope using an 8.0 keV, 0.5 nA, Ar$^+$ primary ion beam rastered over an area of 500 μm x 500 μm with detection of positive secondary ions. With a typical analysis time of 1000 sec the primary ion dose exceeded 1x10$^{15}$ ions/cm$^2$, resulting in complete consumption/fragmentation of the analyte compounds. Working curves were constructed by taking the integrated areas under the decay curves, normalized to the matrix element carbon, and plotting them as a function of analyte concentration in units of grams of compound/grams gelatin matrix.

One of the most severe drawbacks in analyzing compounds in gelatin (and tissue) matrices is the presence of a discrete background arising from polyatomic cluster ions. This background can severely degrade detection limits for lower mass analyte compounds. The influence of this background can be seen in the mass spectrum shown in Figure 1 for a .1 gm/gm (10 wt %) acetylcholine/gelatin mixture. Even for a compound such as acetylcholine, which is considered to have a high ion yield, the detection limit range for the parent cation (m/z 146) was between .001 gm/gm (.1 wt%) and .01 gm/gm (1 wt%). Similar poor detection limits were obtained for other lower mass compounds such as epinephrine and tetrabutyl ammonium bromide.

In general, compounds with higher molecular weights gave better detection limits. Figure 2 shows the integrated signal for the protonated molecular ion of cocaine (m/z 304) vs. concentration. In this case, the detection limit was ~ 100 ppm. High secondary ion yield compounds, such as tetrabutyl and tetrapentyl ammonium bromide, gave detection limits of better than 10 ppm and linear response curves over several decades of concentration. Figure 3 shows the response of the tetrapentyl ammonium cation (m/z 298) vs. concentration (this particular data was acquired using a CCD camera in imaging mode as discussed below). A summary of the preliminary detection limit ranges for the various compounds studied is given in Table 1.

A major difficulty with the analysis of compounds in the gelatin matrix is that the precision is rather poor. Replicate analyses conducted on tetrapentyl ammonium bromide resulted in a typical standard deviation for a single measurement of 20-70%. Because the analytical signal decays rapidly in these experiments, missing the first few channels of the signal decay curve (where the signal is the highest), can introduce a significant error in the area calculation. This is a particular problem when using the ion microscope because there is a noticeable background when the primary ion beam is off the sample. This can result in appreciable damage to the sample between replicate analyses. The result is that initial count rates for the analyte compound generally decreased between subsequent analyses even though supposedly virgin areas of the sample were examined. Preliminary experiments with a ToF SIMS instrument would appear to be promising for improving this particular problem.
appear to give much more precise results. We are currently evaluating the relative merits of the two approaches.

It is interesting to note that there is a saturation in signal for the higher concentrations of the tetrapentyl ammonium bromide which is not seen for the cocaine (preformed molecular ion vs. protonated molecular ion). For tetrapentyl ammonium bromide, the fall off may be due to a higher probability for renutralization with increasing counter ion concentration (a similar saturation in signal has been observed for other tetraalkyl ammonim salts and methylene blue). The observation of molecular matrix effects suggests that care will have to be taken in the interpretation of quantitative data from real samples in which localized analyte molecular concentrations (or localized counter ion concentrations) may result in smaller secondary ion signals than from adjacent regions where the true concentration is actually lower. Clearly, it will be necessary to study these effects in more detail. A summary of preliminary detection limit ranges for the various compounds studied is given in Table 1.

In addition to bulk analysis, the homogeneity of the doped spin-cast films makes them ideal as standards for quantitative molecular imaging SIMS studies. As was shown in Figure 3, using a slow-scan CCD camera, a tetrapentyl/gelatin standard was analyzed using this imaging system as the primary detector. Signal intensities were determined by integrating the total secondary ion counts in each image. Because of its high linearity and high dynamic range, the CCD camera is ideally suited for these studies and linear working curves were obtained over a concentration range of $2 \times 10^{-5}$ gm/gm to .02 gm/gm.

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Detection Limit</th>
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<td>Acetylcholine</td>
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<td>Tetrabutyl Ammonium</td>
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References:
STATIC SIMS ORGANIC SURFACE ANALYSIS OF ELECTRONIC-GRADE GLASSY MATERIALS

Bryan L. Bentz
David Sarnoff Research Center, CN 5300, Princeton, NJ 08543-5300

Static SIMS studies have been undertaken to probe the molecular structural composition of solid surfaces of several classes of glassy materials used in the electronics industry. Because of their unique thermal, electrical, and mechanical properties, glassy materials are widely used in this industry for a number of varied applications, such as panels in display devices and as dielectrics in integrated circuit metal systems.

Low dose SIMS experiments of these insulator-type materials were conducted using a picoampere level, 3 keV neutral primary beam (xenon) on the triple stage quadrupole-based static SIMS instrument developed at the David Sarnoff Research Center. Mass spectral data were acquired over a one minute accumulation time. Charging of the samples did not appear to be severe in our experiments.

1000Å films of methyl polysiloxane type spin-on-glasses (SOG), used in dielectric planarization schemes, were studied to determine the identity and quantity of organics in solid films prepared using various conditions of heat curing. The SOG, once deposited and cured, has electrical characteristics very much like SiO₂. Large differences in the apparent methyl (-CH₃) content of the films were observed by SIMS among films cured under different conditions. Additionally, we studied so-called plasma converted SOG films. Data illustrating some of our findings from these experiments are presented in the table shown below. The SIMS data suggest that the increase in film dielectric strength upon oxygen plasma treatment is related to an increase in the hydroxyl and carbon (hydrocarbon) content of the films.

Work using derivatization reagents is currently underway to investigate the extent of hydroxyl/silanol groups on the surface of spin-on-glasses. For example, Figure 1 presents static SIMS spectra showing the effect of exposing a film of cured spin-on-glass to vapor of hexamethyldisilazane (HMDS). Results obtained to date indicate that the monolayer surface sensitivity and molecular specificity of static Organic SIMS can be employed in surface derivatization schemes to characterize the surfaces of spin-on-glasses.

### SPIN-ON-GLASS FILMS: OXYGEN POST-PROCESSING

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<th>SAMPLE</th>
<th>Ratio O'/OH⁺</th>
<th>Rel. Int., m/z 18, H₂O⁺</th>
<th>Ratio Si⁺/CH₃⁺</th>
<th>Subsurface Ratio O'/OH⁺</th>
<th>Subsurface Ratio SiO⁺/SiO⁺⁺</th>
<th>Dielectric Strength, K</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1, 450°C bake, 60 minutes, No Oxygen exposure</td>
<td>2.7</td>
<td>1.0</td>
<td>4.9</td>
<td>14.8</td>
<td>3.2</td>
<td>3.5</td>
</tr>
<tr>
<td>#2, Same bake, processed in O₂ rf discharge (saw ion bombardment)</td>
<td>2.4</td>
<td>1.9</td>
<td>4.3</td>
<td>14.1</td>
<td>1.1</td>
<td>4.3</td>
</tr>
<tr>
<td>#3, Same bake, processed in O₂ downstream discharge (saw no ion bombardment)</td>
<td>2.1</td>
<td>2.9</td>
<td>3.8</td>
<td>13.3</td>
<td>2.2</td>
<td>6.7</td>
</tr>
</tbody>
</table>

1143
Figure 1

STATIC SIMS ANALYSIS OF SURFACE OF AN SOG FILM
POSITIVE ION DETECTION

Derivatization of Si-OH groups to form Si-O-Si(CH₃)₃
FAB/MS CHARACTERIZATION OF SULFONE ETHER DIAMINE PREPOLYMERS.
John M. Ballard, Hoechst Celanese Research Division, Summit, NJ 07901.

INTRODUCTION
Gel permeation chromatography of some sulfone ether diamine (SED) prepolymers showed that they were mixtures but the molecular weight estimations were not sufficiently accurate to enable the chemical composition of individual components to be determined. Low resolution FAB/MS has provided confirmation of the preparation of the synthetic objectives and larger oligomers.

EXPERIMENTAL
SED prepolymers (provided by Dr. E. Choe) were prepared by the condensation of an aromatic dihydroxy compound (A) with 4,4'-dichlorodiphenylsulfone (B) in a 1:2 molar ratio, followed by treatment with two equivalents of the sodium salt of m-aminophenol (C) to yield the prepolymer. Samples dissolved in m-nitrobenzyl alcohol were ionized by a xenon beam from an Ion Tech atom gun operated at 8keV and 1mA emission current. Positive ion mass spectra (m/z 400-4000/3.6 sec.) were recorded on a VG Trio-3 triple quadrupole mass spectrometer. CAD spectra were generated at a collision energy of 7eV using argon at a pressure of approximately 1 x 10^-9 barr.

RESULTS AND DISCUSSION
When the aromatic dihydroxy compound is 4,4'-biphenol, the synthetic objective (BPSED) has the structure (NH2)CB(AB)C(NH-) with mol.wt. 832.19 for n=1. The full-scan mass spectrum of "BPSED showed an ion series of diminishing intensity at m/z 434, 834, 1233 and 1632. The 400 dalton differences above m/z 834 correspond to the oligomeric series where n=2,3 and 4 respectively, due to incorporation of extra (AB) units. The ion at m/z 434 is assigned to the product where n=0 and indicates the presence of free 4,4'-dichlorodiphenylsulfone in the intermediate (not isolated) product. The data system had centroided some ions at [M+2H]^+ and some at [M+H]^+. However, when limited-scan spectra were acquired at a higher resolution in the MCA mode, the molecular ion regions at m/z 434, 834 and 1233 each gave isotopic profiles in which Peak A is due to the M^+ ion (Fig.1). The observed relative peak intensities are significantly different from those calculated using the given molecular formula. In each case, the observed isotopic pattern is a composite of overlapping patterns due to M^+ and [M+H]^+ ions. The patterns observed at the m/z 432, 832 and 1232 molecular ion regions can be approximated by M^+:[M+H]^+ ratios of 10:7, 5:4 and 5:6, respectively, (Table 1). Although the occurrence of oxidation and reduction processes during FAB/MS has been widely reported (1,2) there was no evidence of enhanced [M+2H]^+ abundance. Similar results were obtained from the prepolymers, 6F-BPASED and BPASED, synthesized using (4,4'-hexafluoro-isopropylidene)-diphenol and 4,4'-isopropyliddenediphenol, respectively as the aromatic dihydroxy compound. The high intensity of the M^+ ions observed here implies a relatively low ionisation potential for these prepolymers (3,4).

MS/MS Experiments
Because of the low absolute intensity of the molecular ion signal, quadrupole Q1 was fully deresolved in order to maximize the transmission of the molecular envelope into the collision cell; the parent ion was therefore a mixture of M^+ and [M+H]^+ together with some 12C and 13C isotopic contributions. CAD spectra were recorded in the MCA mode at a resolution of 200 (at m/z 912) in quadrupole Q3. The m/z 834 molecular ion region of BPSED gave four major ions at m/z 585, 494, 248 and 185 whose
assignments are consistent (within the constraints of low resolution) with the structure of the prepolymer (see Table 4). Similar results were obtained for BPASED and 6F-BPASED.

CONCLUSIONS

FAB/MS and MS/MS has proved effective for the characterization of low-to-medium molecular weight oligomeric mixtures of the type described. A fast-scanning data acquisition under low resolution to maximize signal intensity is adequate for most purposes. The following information can usually be derived: 1) whether the synthetic objective has been prepared, 2) whether intermediates are still present, and 3) whether competing side-reactions have occurred.


<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>DATA FOR BPSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALCULATED ACCURATE MASS FOR C₆H₅Br₂O₂F₂ = 533.11</td>
<td></td>
</tr>
<tr>
<td>MEASURED</td>
<td>RELATIVE</td>
</tr>
<tr>
<td>A</td>
<td>533.23</td>
</tr>
<tr>
<td>B</td>
<td>533.23</td>
</tr>
<tr>
<td>C</td>
<td>533.31</td>
</tr>
<tr>
<td>D</td>
<td>533.28</td>
</tr>
</tbody>
</table>

| CALCULATED ACCURATE MASS FOR C₆H₅Br₂O₂F₂ = 533.19 |
| REL. INT. | CALCULATED |
| FOR 5:1 RATIO m/z (H=Br) |
| A | 533.29 | 100 | 73 | 73 |
| B | 533.19 | 57 | 100 | 100 |
| C | 533.31 | 27 | 47 | 53 |
| D | 533.31 | 9 | 17 | 23 |

| CALCULATED ACCURATE MASS FOR C₆H₅Br₂O₂F₂ = 1322.37 |
| REL. INT. | CALCULATED |
| FOR 3:1 RATIO m/z (H=Br) |
| A | 1328.17 | 100 | 49 | 49 |
| B | 1325.14 | 83 | 100 | 100 |
| C | 1324.09 | 55 | 73 | 75 |
| D | 1325.08 | 23 | 83 | 82 |
| E | 1326.07 | 9 | 22 | 18 |
Abstract

Temperature programmed desorption mass spectrometry (TPD/MS) studies have been performed using organic compounds condensed on a heatable/coolable stainless steel probe in order to learn about their useful lifetimes and stabilities at temperatures and in vacuum environments typically used in fast atom bombardment mass spectrometry (FABMS) of organic samples. Glycerol, 3-nitrobenzyl alcohol, thioglycerol, triethanolamine, and a 3:1 mixture of dithiorthreitol and dithioerythritol ("magic bullet") were chosen for study since they are commonly used as matrix compounds in FABMS.

For all matrix compounds studied, molecular species (M^+ and [M + H]^+) desorb within a relatively narrow temperature range. The desorption peak temperature is unique for each matrix. For some matrix compounds, however, additional species desorb which can only be formed by the dehydrolysis or decomposition of the compound.

These results suggest that for each matrix compound used in FABMS an optimum useful temperature range can be defined. The ultimate performance of each matrix, however, may also be affected by the thermal stability, which varies widely for the compounds studied in this work.
Summary

In this study, several matrix compounds commonly used in FABMS analysis of organic compounds have been studied using TPD/MS. The TPD/MS technique (1,2) was used to obtain information on the stability and lifetime of these matrices under conditions similar to those used in FABMS analysis (3-5).

For some matrix compounds (for instance, glycerol, thioglycerol, and triethanolamine) dehydrolysis to form water and/or decomposition to form methanol and/or ethanol can be significant. This degradation can occur at the same temperature as that typically used in FABMS, thus interfering with the performance of the matrix. The degree of degradation can be ranked as follows:

glycerol = thioglycerol > triethanolamine > dithiothreitol/dithioerythritol
> nitrobenzyl alcohol

Thus, nitrobenzyl alcohol would be the best choice as a FABMS matrix material on the basis of its stability.

Both nitrobenzyl alcohol and thioglycerol desorb around ambient temperature (25-50°C). Glycerol and magic bullet desorb slightly above ambient temperature, while triethanolamine desorbs significantly above ambient temperature. This variability in the desorption temperatures implies that the optimum useful temperature range for each matrix compound is unique, and thus requires the ability to control the sample temperature.

References
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

THE MEASUREMENT OF WORK FUNCTIONS OF SEVERAL ALKALI ALUMINOSILICATES IN VARIOUS CHEMICAL ENVIRONMENTS

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Department of Chemistry, Wright State University, Dayton, OH 45435

The immediate goal of this work was to determine the work functions of several alkali aluminosilicates in various chemical environments. These results will aid in our laboratory's long term objective of better understanding the nitrogen/phosphorus detector (NPD) used in gas chromatography. Presently, the mechanism of this detector is not clearly understood. Several key points about the detector include:

- the heart of the detector is a heated alkali aluminosilicate bead;
- the detector gives selective responses for nitrogen and phosphorus compounds;
- the flow rates of air and hydrogen are such that a true flame does not exist, and;
- the detector can detect both positive and negatively charged species.

The ionization mechanism of the NPD has yet to be fully established. Although a number of mechanisms have been proposed, they do not take into consideration all of the key points mentioned above. It is the opinion of our laboratory that the operation of the NPD is based primarily on empirical knowledge. We feel that if the mechanism of the detector was better understood improvements could be made to increase its sensitivity and selectivity.

Gas chromatographic results were obtained using a Varian 6000 gas chromatograph equipped with a NPD. The injection and column temperatures were optimized for each compound. The carrier gas flow rate was held constant at 30 mL/min of helium. The air flow rate was 180 mL/min and the hydrogen pressure was 17 psi. These conditions were determined to be the optimum for this particular NPD bead. The detector temperature was 300°C.

The work function measurements were made in a test chamber (constructed in-house) that was attached to a vacuum system. The alkali aluminosilicate was made by mixing 0.1 mole of an alkali sulfate with enough refractory cement (Dylon Industries, Grade C-10) to produce 10 grams of material. A slurry (in water) of this mixture was prepared and coated on a tungsten filament. A new filament with the same physical characteristics was used for each material. The size and shape of the coated filament was similar to those used in the NPD. An initial heating of the coated filament was done using a heat gun. Final conditioning consisted of spot-welding the coated filament to the leads of the test chamber and attaching the chamber to the vacuum system. After approximately 12 hours, the alkali aluminosilicate was conditioned by slowly heating the filament over a 4 hour period up to a temperature of 1000°C.

The emission current was measured at a pressure of 0.10 mtorr in the absence of any added sample and 220 mtorr in the presence of selected nitrogen and phosphorus compounds. The emission current was collected and measured using an electrometer. The temperature of the alkali aluminosilicate was measured using an infrared thermometer (Omega Engineering model OS1200-HAC-4-B) which was equipped with a close focus option. The emissivity was set at 0.90 which is typical of most ceramics having the same characteristics.

The following table contains an abbreviated list of nitrogen compounds used in both the NPD and the test chamber. Plotting emission current (i) as a function of 1/temperature should give a line with a slope related to the work function of the bead material. We do not make an attempt in this study to measure the work function exactly, instead we are interested in the relative changes in the slope of these plots. In this table the compounds have been normalized. The numbers in brackets under the NPD data are the response factors obtained at a bead temperature of 530°C and 675°C, respectively.

It is important to note that the relative changes in each experiment stays the same. This suggests that in both processes a similar change in work function occurs. This change, as indicated from the test chamber results, is a decrease in work function which would promote the generation of negatively charged species.

An interesting question arises concerning the need for the hydrogen/air environment for a response to be observed in the NPD. Obviously this environment is lacking in the test chamber.
under vacuum conditions. Comparing the absolute current produced at various bead temperatures suggests a possible explanation. The quantity of analyte present in the vacuum test chamber is approximately seven orders of magnitude higher than that introduced into the NPD. Even though this is true, the ion current produced in the NPD is several orders of magnitude higher than in the test chamber at approximately the same bead temperature. This suggests that the hydrogen/air environment may be necessary to promote gas phase decomposition of the analyte. In addition, the parallel behavior in work function changes indicates that ionization is most likely a surface process.

Phosphorus compounds behave differently than nitrogen compounds. From the following figure the curve for trimethyl phosphate (TMP) by itself in the test chamber indicates the work function actually increases. When air is introduced (bringing the total pressure up to 500 mtorr) the work function decreases. The behavior of phosphorus compounds in the NPD indicates a strong interaction with the surface of the alkali aluminosilicate bead even in the presence of an air/hydrogen environment. In the absence of this environment within the test chamber, phosphorus compounds may chemically alter the surface. Upon the addition of air, whatever process taking place is hindered. From this behavior we cannot rule out that decomposition of an analyte in the presence of air/hydrogen can take place on the surface of the alkali aluminosilicate material. These decomposition products may lower the work function which favors the formation of negatively charged species.

To date, our results indicate that the nitrogen/phosphorus compound may decompose either in the gas phase or on the surface of the hot bead. It appears that for most nitrogen compounds decomposition may most likely occur in the gas phase whereas for phosphorus compounds the surface plays a major role. For both nitrogen and phosphorus compounds the adsorption of decomposition products lowers the work function which favors the ionization of these species.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>NPD</th>
<th>TEST CHAMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>NITROPROPANE</td>
<td>1.94</td>
<td>1.11</td>
</tr>
<tr>
<td>PYRIDINE</td>
<td>2.23</td>
<td>1.24</td>
</tr>
<tr>
<td>OCTYLAMINE</td>
<td>2.41</td>
<td>1.36</td>
</tr>
<tr>
<td>HEPTYL CYANIDE</td>
<td>2.66</td>
<td>1.54</td>
</tr>
<tr>
<td>HEPTYLAMINE</td>
<td>2.25</td>
<td>1.31</td>
</tr>
<tr>
<td>AMYLAMINE</td>
<td>2.51</td>
<td>1.45</td>
</tr>
<tr>
<td>BENZYL CYANIDE</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>t-BUTYLAMINE</td>
<td>2.25</td>
<td>1.33</td>
</tr>
</tbody>
</table>

To date, our results indicate that the nitrogen/phosphorus compound may decompose either in the gas phase or on the surface of the hot bead. It appears that for most nitrogen compounds decomposition may most likely occur in the gas phase whereas for phosphorus compounds the surface plays a major role. For both nitrogen and phosphorus compounds the adsorption of decomposition products lowers the work function which favors the ionization of these species.
Reduction of Mass Bias Effects in Inductively Coupled Mass Spectrometer Systems


Inductively coupled plasma mass spectrometry (1,2) has become a widely used method for the determination of elemental concentrations in solutions. A wide range of elements may be covered by the technique, and detection limits in many cases are in the parts per trillion range by weight. Samples are introduced into an argon inductively coupled plasma as a fine aerosol, and ions produced in the plasma are transferred into an analysing mass spectrometer via an interface which normally consists of a series of pumps and apertures which are followed by a Bessel Box type energy analyser and matching optics. This arrangement is shown in figure 1.

Plotted on this diagram are the trajectories of ions of three different energies as calculated using SIMION (3). Energies of 0.5, 2, 12 volts have been chosen since those correspond to the energies of ions of mass 10, 40, 238 which are assumed to pass through the skimmer orifice with a velocity of $3 \times 10^5$ cm/sec. It can be seen that focusing of the ions of different energies varies considerably according to energy. This means that there is a substantial mass bias introduced by this form of transfer optics. A further effect which must be considered is the space charge spreading of the ion beam as it travels through the low voltage region of space behind the skimmer. Since all ions are travelling with approximately the same axial velocity, and transverse acceleration $\frac{e}{m}$ spreading will be greatest for those ions of lightest mass.

Again, this can be modelled by SIMION by calculating the space charge potential due to a column of charge emanating from the rear of the skimmer cone. The results of this calculation are shown in figure 2. Again the overall effect is to produce a significant mass bias for the transfer function into the mass analyser.

The object of the centre stop in the lens system is not only to function as the central part of the Bessel filter, but also to stop photons and neutral particles travelling into the collector region of the mass filter and producing an undesirable background signal. An arrangement which also produces the required blocking features is shown in figure 3.
The plasma/vacuum interface is treated as a conventional ion source (such as a duoplasmatron) and a conical acceleration electrode is used immediately behind the skimmer cone. A voltage of typically 2kV-ve is used on this element, whilst focusing and deflection are controlled by subsequent components of the ion optical system. Trajectories are shown again for ions having energies of 0.5, 2, 12 volts, and it can be seen that the chromatic properties of this transfer lens are very much better than those of figure 1. Space charge effects are also minimised using the high voltage transfer lens approach. Direct line of sight from the sampling interface into the quadrupole is avoided, whilst the central part of the ion beam, previously blocked off by the Bessel Box arrangement, is available for mass analysis. The resulting system has:

a) LOW MASS BIAS  
b) HIGH TRANSMISSION  
c) LOW BACKGROUND

The mass bias as a function of mass is shown in figure 4 and is compared with that of a Bessel Box type transfer system.

![Figure 4](image)

Spectra from and detection limits for dilute solutions of certain elements are given in figure 5.

![Figure 5](image)

Detection Limits

| Element | Limit  
|---------|--------
| Mg      | $< 0.06$ ppt 
| Cd      | $< 0.4$ ppt 
| Pb      | $< 0.3$ ppt 
| Bi      | $< 0.5$ ppt 
| U       | $< 0.6$ ppt |

References.

AUTOMATED ANALYSIS OF \(^{13}\text{N}\) and \(^{12}\text{C}\) FROM THE SAME SAMPLE: A New Interface for Stable Isotope Ratio MS

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The variation of isotope abundances in nature is small. For \(^{12}\text{C}\) and \(^{13}\text{N}\) in particular, any method to study this variation needs a measurement precision of the order of 0.1 permil (8-notation: 
\[ \delta = \left( \frac{R_{\text{Sample}}}{R_{\text{Reference}}} - 1 \right) \times 10^6 \].

For \(^{12}\text{C}\) this corresponds to \(10^{-4}\) at% or 1 ppm, for \(^{13}\text{N}\) the values are \(4\times10^{-8}\) at% or 0.4 ppm. Such precision can be obtained with specialised Isotope Ratio Mass Spectrometers. These offer simultaneous ion current detection with multiple Faraday cup assemblies, high current ion sources and gas switching with a "Changeover valve". For all isotope measurements samples must be converted to clean \(\text{CO}_2\) and \(\text{N}_2\) gas respectively. These sample gases are measured against reference \(\text{CO}_2\) and \(\text{N}_2\) gases of known isotopic composition. The latter are calibrated versus international standards.

Figure 1 shows a schematic overview of the interface for Carbon and Nitrogen analysis (CTBOX CN). Milligram size samples are combusted in a commercial CHN analyser. The product gases \(\text{CO}_2\), \(\text{H}_2\text{O}\) and \(\text{N}_2\) are swept into the interface without splitting. Automated cold traps designed for fast and reproducible temperature changes between -196°C and +150°C separate the gases from the Helium carrier and from each other. The CTBOX CN also acts as inlet system to the mass spectrometer. \(\text{N}_2\) and \(\text{CO}_2\) are measured from separate compartments with dedicated capillaries to the changeover valve. The latter has a new design with four ports. Each of the ports accepts a capillary from a gas reservoir. Two of the reservoirs contain the reference \(\text{CO}_2\) and \(\text{N}_2\) gases while the other two are connected to the CTBOX CN interface. Only one of the ports at a time may communicate gas to the MS, the other three being switched to the waste line pump.

Figure 1 Schematic outline of Trapping Interface CTBOX CN
The whole system is software controlled. Nitrogen and Carbon from a single combustion are analysed in sequence:

**Nitrogen:**
After trapping in the first N₂ trap with the aid of 5 Å Angstrom zeolite (Alltech) at -196°C the trap is heated to 90°C for quantitative release of N₂. PM2 measures the sample amount. The sample then is quantitatively transferred to the second volume (500µl) which is filled with a small amount of silica gel (Fischer). Depending on the previous PM2 reading the gas is measured from this small volume or it is back expanded into the volume between the two preceding valves after heating. For fast isotopic equilibration between the silica gel and the gas volume we added a mixing cycle by cooling and heating the trap one more time with different times and temperatures. Omitting this cycle leads to a measurable dependence of the δ-value upon signal height (=0.1 permil/nA). Care must be taken during all times that the absorbents in the N₂ traps are not contaminated with CO₂. Even small amounts will spoil the N₂ results due to the fact that CO⁺ formed from CO₂ in the ion source has a ²⁹/²⁸ ratio ≠600 permil different from natural abundance Nitrogen.

**Carbondioxide:**
CO₂ is first trapped from the Helium carrier stream in the first trap at liquid Nitrogen temperature together with water. After pumping He and heating the trap to -80°C CO₂ is distilled to the second trap where it's amount is measured after heating. This procedure ensures that no trace of CO₂ may enter the nitrogen trap. Depending on the PM1 reading, the CO₂ is either measured directly when the Nitrogen analysis is finished or it is further concentrated into the small volume cold trap (250µl) preceding the CO₂ capillary. From here, reexpansion is employed for intermediate size samples.

**Results:**
Figure 2 presents some of the data obtained with the CTBOX CN interface. Especially for small samples (<28µmol) the blank must be controlled precisely for good Nitrogen measurements. The results demonstrate that good agreement is observed for international standards between the expected and the observed values. Furthermore, this agreement is true independent of sample size (after blank correction) for samples in the lower µmol N and C range. Cross contamination between CO₂ and N₂ is negligible. CO₂ formation in the combustion is not observed.

![](image.png)

**Figure 2** C&N isotope analysis on a mixture of N₁/N₂ with NBS21

<table>
<thead>
<tr>
<th>C &amp; N Isotope Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ PDB</td>
</tr>
<tr>
<td>𝛥₁₅ᵃᵢʳ</td>
</tr>
<tr>
<td>15 µmol C</td>
</tr>
<tr>
<td>mg sample</td>
</tr>
</tbody>
</table>

1154
DETECTION OF NONCOVALENT ENZYME-SUBSTRATE AND RECEPTOR-LIGAND COMPLEXES BY CONTINUOUS-INTRODUCTION ION SPRAY MASS SPECTROMETRY.

Jack Henion* and Yu-Tsyr Li, Drug Testing and Toxicology, New York State College of Veterinary Medicine, Cornell University, 925 Warren Drive, Ithaca, New York 14850 USA, and Bruce Ganem, Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, New York 14853 USA

There are few methods known for detecting and identifying enzyme-substrate, receptor-ligand and antibody-antigen complexes whose weak, noncovalent interactions constitute the essential basis of molecular recognition in the biological world. We report the use of mass spectrometry (MS) to detect non-covalent enzyme-substrate and receptor-ligand complexes as their protonated, multiply charged species. The enzymatic reaction of egg-white lysozyme (HEWL, MW 14,305) with various substrates and the cytoplasmic receptor, FK binding protein (FKBP, MW 11,776 Da) with various drugs (FK506, MW 804 Da; rapamycin, MW 913 Da; cyclosporin A, MW 1202) were monitored using continuous introduction ion spray MS in real time under physiological conditions (HgO, pH 5.0, and 7.5, respectively) in aqueous acetate buffer. That the observed complexes were not anomalous aggregates resulting from the electrospray ionization process was supported by several lines of evidence. Results from on-line monitoring of the interaction of lysozyme and FKBP with carefully chosen substrates are described that demonstrate mass spectrometric detection of transient complexes. These studies suggest mass spectrometry may be a powerful tool for characterizing noncovalent enzyme-substrate and receptor-ligand complexes.

EXPERIMENTAL

All enzyme-substrate mixtures were introduced in 100% aqueous buffered solutions by continuous infusion of the sample (2μL/min) at ambient temperature through an ion spray interface. The mass spectrometer was a Sciex TAGA 6000E triple quadrupole system upgraded to an API III operated in the single MS mode. Enzyme-substrate ratios of lysozyme-NAG were typically 1:13.9 at a pH of 5.0, and receptor-ligand ratios were 1:1.6 for FKBP:drug at pH 7.5. The mass spectrometer was tuned and mass calibrated on the enzyme and receptor, respectively, and acquisition experiments utilized 2 sec scans with 10-20 scans summed.

RESULTS AND DISCUSSION

For the HEWL studies the substrates included NAG, NAG2, NAG3, NAG4, and NAG6 in addition to TACL in addition to mixtures of these. One, ten and sixty minute infusion experiments that studied the hydrolysis of NAG6 to NAG4 by HEWL clearly showed rapid hydrolysis of NAG6 with the production of NAG4. Both appeared as a complex with the (M+H)+ charge state of HEWL. Related studies with measurements taken 10 min after commencing the infusion experiments showed varying degrees of complex formation between HEWL and NAG4 showing the most abundant ion current signal as predicted from known solution behavior. When HEWL was mixed with TACL, a known inhibitor of the hydrolysis the HEWL-TACL complex was detected with significant reduction of the HEWL-NAG4 complex. Finally, when an equimolar mixture of NAG3 or NAG6 and TACL were subjected to the same experimental conditions, the HEWL-TACL complex predominated in each case further suggesting that an active site-directed interaction plays a role in the formation of the enzyme-substrate species detected in this work.

Additional competition experiments with an important receptor, FKBP and the immunosuppressive drugs, FK 506, rapamycin and cyclosporin A (CsA) were conducted. These experiments were carried out in the same manner as described for HEWL. A competition experiment in which FKBP was combined with equimolar amounts of FK 506 and rapamycin clearly showed the presence of both receptor-ligand complexes at m/z 1803.1 and 1821.0, respectively, in the ion spray mass spectrum (Figure 1). Peak integration revealed that the rapamycin complex was twice as abundant as the FK 506 complex, in striking agreement with known k4 values. This result indicates that noncovalently bound species with suitably high association constants can be detected directly in a complex mixture without prior chromatographic separation. Ion spray MS might thus simplify the characterization of such structures when either the host or guest MW is known.

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In a broader context, the use of MS to detect and identify molecular association complexes resulting from the interaction of charged macromolecules with their specific molecular ligands (or vice-versa) may find additional applications in two other classes of biologically important Noncovalent association complexes. The interactions of cell surface receptors with specific effector molecules and extracellular components play critical roles in signal transduction, cellular adhesiveness, and many other multicellular processes. Antibody-antigen recognition and aggregation phenomena are central to the function of the vertebrate immune system, and may likewise be amenable to study using antibody Fab fragments, single chain V_{L}/V_{H} and V_{H} antigen-binding proteins, as well as other, lower-MW immunoglobulins.

ACKNOWLEDGMENTS

This research was supported by a grant from the Cornell Biotechnology Program, which is sponsored by the New York State Science and Technology Foundation, a consortium of industries, the U.S. Army Research Office, and the National Science Foundation. We thank Professor Taichi Usui for providing NAQe and Professor Gustav Lienhard for providing TACL. We also thank Professor Stuart Schreiber (Harvard University) for providing generous samples of FKBP, FK 506, rapamycin and cyclosporin A.
Structural Characterization of Proteins using Open Tubular Liquid Chromatography Coupled with Electrospray Ionization on a Triple Quadrupole Mass Spectrometer

Patrick R. Griffin, Leroy E. Hood, and John R. Yates, III

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The focus of the mass spectrometry group at Caltech has been the development of new approaches for the analysis of biomolecules. Presently in our laboratory we have three mass spectrometers; a triple quadrupole (Finnigan TSQ-700) with LSIMS and electrospray, a quadrupole Ion Trap with an electrospray ion source, and a time-of-flight instrument for matrix-assisted laser desorption mass spectrometry. We have developed methods for protein sequence analysis based on coupling liquid chromatography with electrospray ionization. The methodology presented in this paper offers a high sensitivity, high throughput strategy for analyzing the primary structure of proteins.

Using electrospray ionization on the TSQ-700 a strong signal is obtained when infusing a 5 pmol/ml solution of angiotensin. The same intense signal can be obtained when lowering the flow rate to as low as 100 nl/min allowing less material to be consumed during the analysis. For instance, scanning for 1 min at a flow rate of 1 μl/min consumes 5 pmol of sample (for a 5 pmol/μl solution). Scanning for 1 min at a flow rate of 100 nl/min consumes 500 fmol of sample (1 order of magnitude less material needed for analysis). To take advantage of this observation, we have been investigating the use of open tubular liquid chromatography coupled to electrospray tandem mass spectrometry. Using OTLC-MS, molecular masses of peptides could be recorded loading on as little as 100 fmol of sample. Isocratic OTLC allowed MS and MS/MS experiments to be performed on 500 fmol of a synthetic peptide in a single run. The methodology presented in this paper offers a high sensitivity, high throughput strategy for analyzing the primary structure of proteins.

C18 derivatized fused silica columns can be made using a stainless steel pressure vessel (Figure 1) and the following conditions.

1. Pass 500 μl of 6 N HCl through a 1 m x 50 μm length of fused silica capillary.
2. Pass dry N2 through the capillary for several minutes.
3. Pass 500 μl of a 5% solution of octyldecyltrichlorosilane in hexane through the capillary.
4. Pass 500 μl of hexane through the capillary.
5. Bake the column at 130 C for 1 h.
6. Pass 500 μl of 0.1% TFA through.
7. Load sample and wash with 200 μl of 0.1% TFA.

Shown in Figure 2 is the reconstructed ion chromatogram for the analysis of 100 fmol of angiotensin by OTLC-MS. 100 fmol of angiotensin was loaded onto a C18 derivatized fused silica capillary column and unbound material was washed off with 0.1% TFA. The peptide was then eluted isocratically with 10% acetonitrile in 0.1% TFA at a flow rate of 200 nl/min. The electrospray mass spectrum from this analysis is displayed in Figure 3.

The same experiment was performed on 500 fmol of a synthetic peptide of structure SPYVESEDAPPQK. The sample was then eluted isocratically into the electrospray ion source using 10% acetonitrile in 0.1% TFA at a flow rate of 250 nl/min. The peptide elutes over several scans thus allowing MS and MS/MS experiments to be performed with a single sample loading. The RIC from this analysis is shown in Figure 4. The electrospray ionization MS and MS/MS spectra are displayed in Figures 5 and 6, respectively.

OTLC-MS can be used for analyzing proteins as is demonstrated in Figure 7. The Reconstructed Ion Chromatogram is from an analysis of 2 pmol of a novel post synaptic neurotoxin, β-bungarotoxin, by OTLC-MS.

Acknowledgements: Finnigan MAT, and NSF Science and Technology Center.
Characterization of Antibodies and Other Large Glycoproteins up to 200 kDa by Ionspray Mass Spectrometry

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Antibodies (immunoglobulins) are a class of large biomolecules responsible for the functioning of the immune system. The average molecular weight (MW) of four intact IgG class monoclonal antibodies (Fig. 1), namely anti-(α1-antitrypsin) (148,484±4 Da), anti-(α1-acid glycoprotein) (149,599±12 Da), anti-(β-galactosidase) (two components, I 150,544±10 and II 151,496±17 Da) and anti-RNA (150,475±11 Da), were determined using an ionspray mass spectrometer (Sciex API III triple quadrupole MS). It is very likely that the MW differences between the experimental MWs and the estimated IgG sequence MW (~140 kDa) are mainly due to the sugar attachments on the Fc domains of the antibodies' heavy chains. Still under investigation is whether the MW difference of 952117 Da between the two components of anti-(β-galactosidase) (Fig. 1c) are caused by their difference in the sugar content or in the genetic coding of the polypeptide chains.

Chemical disintegration of the anti-RNA IgG by reducing the inter-chain disulfide bridges (S-S bonds) resulted in one pair of light chains and one pair of heavy chains (the two in each pair are identical). The determined MW of light chain (24,106.9±0.4 Da) and heavy chain (heterogeneous, 30% of 51,021.5±0.8 Da and 70% of 51,184.0±0.9 Da) not only revealed the sizes of the constituent chains, but also confirmed the average MW determined for the intact antibody. Collisionally activated dissociation (CAD) of the 150 kDa antibody ions, at both the ionspray interface region and the MS/MS collision chamber in Q2 region without MS-1 m/z separation, produced a charge distribution profile corresponding to the light chain (~24 kDa) (see Fig. 2 for the total ion CAD spectra of anti-(α1-acid glycoprotein) taken under different collision energies at Q2). Apparently, the collision energy intake in a quadrupole type instrument is sufficient for the CAD of multiply charged macro-molecular ions as high as 150 kDa. However, the heavy chain fragments were not observed; the reason is still not clear.

Although exactly equal moles of light (24.1 kDa) and heavy (51.1 kDa) chains were obtained upon reduction of the inter-chain disulfide bonds of the anti-RNA IgG, the observed MS signals for the light chain was >10 times bigger than those for the heavy chain. Reduction of the anti-RNA Fab fragment (47.6 kDa) from papain digestion also yielded equal moles of the intact light chain and the shortened heavy chain (23.5 kDa). The light chain, which was slightly heavier by now, however, again gave 9-fold stronger signals than the shortened heavy chain. Therefore, serious attention must be given to the relative sensitivities of the species in a mixture, if one is to use ionspray or electrospray technique for their quantitation.

Human serum transferrin (HST), a glycoprotein serving for iron transportation in blood, gave a MW of 79,556.8±1.7 Da (Fig. 3a). The value is 4,414 Da higher than the calculated sequence MW (75,142.9 Da), indicating the attachment of ~23 sugar units in this protein via post-translational modification. The charge distribution profile of HST is sharply different from that of bovine serum transferrin (BST; three components, I 78,030.5±1.8, II 78,326.8±3.3 and III ~78,609 Da, see Fig. 3b), implicating that the difference in glycosylation may affect protein charging efficiency in solution.

The single chain subunit of human α2-macroglobulin (a glycoprotein whose tetrameric aggregate serves as an inhibitor to nearly all endo-proteinases by trapping them inside the gigantic molecular cage) yielded a MW of 186,070±150 Da (Fig. 4a). This measured MW is 25.2 kDa higher than the sequence MW (160,837 Da, reduced form). Therefore, post-translational modifications make up 13.6% of the total protein mass, consistent with the previous biochemical studies.

Human complement C4, a glycoprotein and one of the key complement components responsible for the destruction of alien cells and viruses, gave a MW of 196,863±29 kDa (Fig. 4b), in agreement with the gel electrophoresis estimate (~200 kDa). The MW difference of 9.8 kDa between the observed whole MW and the preliminary sequence MW (~187,087 Da) reflects the sugar content of ~5% in this protein, in line with the reported value of ~7%.

The above studies demonstrate that ionspray MS is capable of accurate MW determination for macro-biomolecules up to 200 kDa. The MW difference between the experimental and sequence MW values can be used to determine the degree of post-translational modifications in a protein. MS/MS analysis is feasible for multi-charged macro-ions as large as 150 kDa. The relative sensitivities of the constituent components must be determined to quantitate proteins in a mixture.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Fig. 1

Mass Spectra of Antibodies

Antibody (a) Myeloma protein
MW 146,886±15 Da

Antibody (b) Anti-<i>γ</i>-globulin
MW 148,894±15 Da

Antibody (c) Anti-light chain
MW 148,894±15 Da

Antibody (d) Anti-heavy chain
MW 150,475±11 Da

Fig. 2

Total Ion CAD Spectra of Antibody

- **80 V**
  - Light Chain Fragment
  - MW 23,722±13 Da

- **130 V**

- **160 V**

Fig. 3

Fig. 4
Characterization of Oligodeoxynucleotide Conjugates by Electrospray Ionization Mass Spectrometry

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Synthetic oligodeoxynucleotides are important components in many molecular biology experiments. They may be probes for cDNA hybridization experiments, primers for PCR reactions, or pieces of synthetic genes for site-directed mutagenesis studies. Oligomers that are conjugated to a labeling reagent, such as N-hydroxysuccinimide-biotin or fluoresceinyl-isothiocyanate, are now commonly used in many hybridization experiments for DNA detection, sequencing, and diagnostic applications. Also, nuclease resistant oligodeoxynucleotide analogs and derivatives containing cross-linking or cell-targeting moieties are under study as therapeutic agents. At present, few methods exist for assaying proper synthesis, deprotection, and conjugation of these molecules. Oligodeoxynucleotides are a class of molecules that, except for oligomers smaller than n=10, have only recently become amenable to routine analysis by mass spectrometry as a result of the introduction of new ionization techniques. Recent experiments have shown that oligodeoxynucleotides may be analyzed readily by electrospray ionization (ESI) mass spectrometry (1-3). In most cases, however, the quality of the spectra have been limited by the presence of sodium adducts of the molecular ion species. The sodium is introduced during the electrophoretic purification of the crude oligomers from aqueous buffer components. The adducts become a severe problem in the spectra of larger oligomers, where the number of added sodium atoms in each peak cannot be determined, or the resolving power of the instrumentation is insufficient to separate the different adduct peaks. In addition, the adducts spread the available ion current over several different peaks, thereby reducing the signal intensity.

We describe here a rapid means of improving the mass spectrum of oligonucleotides and their conjugates by replacement of sodium with ammonium ions to reduce adduct formation. These samples show greatly improved spectra by electrospray ionization and extend the useful mass range for analyzing oligodeoxynucleotides beyond 20 kDa.

**EXPERIMENTAL**

*Synthesis of oligodeoxynucleotides* -- Oligodeoxynucleotides were synthesized using H-phosphonate (Milligen Biosearch 8600 DNA Synthesizer) or phosphoramidite (Milligen 7500 DNA Synthesizer) methodologies. Reaction products were examined and purified by electrophoresis (10-15% polyacrylamide gel, 7 M urea, visualization by UV shadowing).

*Sample preparation for mass spectrometry* -- Purified oligomers (100 μl, ~5 OD260/ml) were treated with ammonium acetate (10 M, 34 μl), precipitated with 2.5 vol. cold ethanol, pelletted, and washed with cold 70% ethanol. This quantity is 1-2 nmol of a 30-mer. The precipitated oligomers were reconstituted in water to a concentration of 50-200 pmol/μl.

*Mass spectrometry* -- Immediately before analysis, a 10 μl aliquot of the oligomer was added to 5 μl of acetonitrile and 5 μl of buffer. The buffers used were 10 mM ammonium acetate or ammonium hydroxide. The samples were infused at 2 μl/min with a Harvard Apparatus syringe pump into the ionspray interface of a Sciex API III triple quadrupole mass spectrometer, operated in the negative ion mode.

**RESULTS**

The ESI mass spectrum of a typical 30-mer, before removal of sodium, is shown in Fig. 1. The inset is the largest peak expanded to show the individual sodium adduct ions. The peak at m/z 944.2 corresponds to [M-27H+17Na+]10+. The same oligomer, following ammonium precipitation, is shown in Fig. 2. The peaks are much sharper, and the largest peak in each cluster contains no sodium atoms. The charge distribution is changed only modestly from Fig. 1, with the [M-12H+]12+ peak as the most abundant ion.

Other conjugated oligomers also produce sharp, well separated peaks when analyzed by electrospray ionization mass spectrometry. Figure 3 shows the spectrum of a biotin-conjugated 20-mer. The largest peak in each cluster includes no sodium atoms. Electrospray ionization mass spectra of other oligomers as large as 77-mers have been obtained successfully following the
The ability to rapidly obtain masses accurate to ±1-2 Da for synthetic oligodeoxynucleotides of most sizes makes mass spectrometry a valuable tool for the nucleic acid chemist, just as it has become an established technique for analyzing synthetic peptides.

ELECTROSPRAY IONIZATION MASS SPECTROMETRY OF WATER AND HIGHLY CONDUCTIVE AQUEOUS SOLUTIONS. Swapan K. Chowdhury and Brian T. Chait, The Rockefeller University, New York, N.Y. 10021.

The electrospray ionization mass spectrometry of water, acidified water, and aqueous protein solutions not containing organic solvents and without nebulization has been investigated. Aqueous solutions are difficult to electrospray because they have high surface tensions and high conductivities. The high surface tensions (T) normally necessitate the use of high voltages (V) on the spray needle (V ~ T^{1/2}) causing corona discharge in ambient air and destabilization of the spray. It has been demonstrated in the present investigation that purely aqueous solutions can be electrosprayed by the use of sharpened capillary needle tips. The sharp tips allow electrospray of aqueous solutions to occur at reduced electric fields, thus avoiding corona discharge destabilization. This observation is in accord with the prediction of Smith, who points out that V ~ T^{1/2}/2ln(4h/r); where r = o.d. of the spray needle and h is the tip to counter electrode distance. Thus for a constant T and h (5 mm) a change of capillary o.d from 700 μm to 200 μm decreases the required onset potential (V) by a factor of 1.4. The spray conditions as a function of spray voltage and flow rate for the electrospray of water is given in Table 1.

The electrospray of water or water containing small amounts of acid or other electrolytes provides an intense source of large ionic clusters of water molecules. The cluster size can be controlled by varying the temperature of the capillary tube used to transport the ions produced by electrospray into the vacuum system of the mass spectrometer. Under one set of experimental conditions, the electrospray ionization mass spectrum of 1% aqueous acetic acid exhibits protonated water cluster ions containing from 6 to 52 water molecules. The cluster ions, H^+(H_2O)_n, with n=21, 28, 33, 35, 37, and 42 appear to have special stability. It has been postulated by many investigators that the (H_2O)_{21}H^+ ion has a special geometry: the pentagonal dodecahedron with the oxygen atoms of 20 water molecules situated at the corners and a H_2O ion trapped inside the dodecahedron cage. To our knowledge, unassisted electrospray ionization mass spectra of aqueous solutions without the addition of any organic solvent have not been previously reported in the literature.

The ability to electrospray aqueous protein solutions without organic solvents allows the mass spectrometric investigation of conformational changes of proteins in solution and the investigation of the role of solvent composition on the mass spectrometric sensitivity of protein ions. Shown in Fig. 3 are the electrospray ionization mass spectra of horse heart cytochrome c obtained from aqueous acetic acid solutions of pH 2.6 (Fig. 3a) and 3.8 (Fig. 3b). In the top spectrum, ions are produced with a single charge state distribution with charges ranging from 18 to 11. In contrast, the bottom spectrum (Fig. 3b) exhibits ions with two discrete distributions of charge states. One distribution (also observed in the top spectrum) centers around 14+ and the other ranges from 10+ to 7+, with 8+ being the most intense ion. The formation of two charge state distributions results from two different conformational states of horse cytochrome c. At the lower pH, the majority of the protein is in a highly charged unfolded state (Fig. 3a). When the pH is increased from 2.6 to 3.8 a large fraction of cytochrome c molecules converts to a tighter conformation that can hold on to a lower number of charges.

LITERATURE CITED
### Table I

Spray conditions as a function of spray voltage and flow rate for the electrospray of water.

<table>
<thead>
<tr>
<th>Flow rate (μl/min)</th>
<th>Voltage on the spray needle (kV)</th>
<th>Spray current (nA)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td></td>
<td></td>
<td>No steady spray at any voltage</td>
</tr>
<tr>
<td>1.5</td>
<td>3.20</td>
<td>90</td>
<td>Fluctuating</td>
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<tr>
<td></td>
<td>3.50</td>
<td>350</td>
<td>Steady</td>
</tr>
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<td></td>
<td>3.70</td>
<td>1250</td>
<td>Fluctuating</td>
</tr>
<tr>
<td>1.0</td>
<td>3.10</td>
<td>-</td>
<td>No spray</td>
</tr>
<tr>
<td></td>
<td>3.17</td>
<td>20</td>
<td>Steady</td>
</tr>
<tr>
<td></td>
<td>3.35</td>
<td>65</td>
<td>Fluctuating</td>
</tr>
</tbody>
</table>

The distance between the spray tip and the transport capillary tube was 6.7 mm.

---

**Fig. 1**

**Fig. 2**

**Fig. 3**

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H^+(H_2O)_n
THE EFFECT OF DIFFERENT SOLVENT SYSTEMS ON THE ELECTROSPRAY IONIZATION OF PROTEINS AND GLYCOPROTEINS, AND THE SEPARATION OF PROTEINS USING PACKED SILICA COLUMNS.

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We previously reported an approach to electrospray that requires no counterflowing gas (1), where the ion source and consequently electrospray chamber are maintained at approximately 250°C and 60°C respectively, to effect ionization and desolvation. Spectra from protein mixtures and modest sized proteins such as bovine serum albumin (BSA) at these source temperatures exhibit fully desolvated ions with no thermal decomposition.

This electrospray system is usually operated at a spray chamber temperature of 60°C which allows aqueous solutions to be sprayed with stable ion currents. We find heating the ion source of particular benefit when investigating compounds such as glycoproteins and proteins which have tight tertiary structures, for example bacterial proteases. Such species were found to be insoluble in organic solution (methanol or acetonitrile) at the levels of concentration required for analysis by electrospray (typically 1 to 300 ng/μL). Even when the sample was prepared in aqueous solution then further diluted in organic solvent the sample would precipitate out of solution at generally > 20% organic, whereupon the 100 micron silica line leading to the electrospray probe clogged. Better sensitivity was seen when 2 to 5% acetic acid was added to solution in preference to 0.1% TFA as generally a 25% loss in signal intensity was observed with TFA. However for ribonuclease B we also obtained excellent spectra when no acid was added to the aqueous solution.

The effect of chamber temperature on the electrospray signal generated from infusion at 5 μL/min of an aqueous solution, 5% acetic acid, of proteinase K (SIGMA Cat. No. P5056; Lot. 70H6838) is shown in Figure 1. An impurity measured at 25,206 daltons is seen at lower chamber temperatures (48 to 60°C), with very weak ions for proteinase K observed; too weak to perform a mass determination. However, on increasing the chamber temperature (> 65°C) the proteinase K becomes the predominant signal.

![Figure 1](image-url). Full scan electrospray mass spectra obtained from a 100ng/μL aqueous solution (5% acetic acid) of Proteinase K at a chamber temperatures of 50 and 78°C. Spectra were acquired at 9 secs/scan. Spectra shown are an average of 3 single scans. Determined molecular weight = 28,910.5 ±/− 5 daltons.
A similar but more dramatic increase in signal intensity was observed for the glycoprotein ribonuclease B and to a lesser extent the bacterial protease thermolysin. It is proposed that the presence of heat helps relax the protein structure sufficiently to ionize the basic residues protected in the tight tertiary structure of the bacterial proteases and also helps fully desolvate ions. It should be noted that myoglobin in aqueous solution, whilst requiring chamber temperatures of > 55°C, does not increase dramatically in signal intensity at elevated chamber temperatures, as shown in Figure 2.

**FIGURE 2.** A plot of intensity for the largest peak in the multiply charged envelope vs electrospray chamber temperature, for the compounds shown, when infused at a flow rate of 5 uL/min at the 100 ng/uL level in aqueous solution, 5% acetic acid.

It is suggested that the best conditions in our interface for "difficult" proteins with tight tertiary structures and glycoproteins such as ribonuclease B are a combination of an aqueous buffer system (5% acetic acid) and a higher than normal chamber temperature (70 to 80°C). Under these conditions the accuracy of molecular weight determination is often better than 0.01%.

This electrospray system is particularly suited to the separation of proteins using packed 320 micron i.d. silica columns. These columns require low flow rates, typically 3.0 to 10.0 microlitres/min, where the electrospray affords the highest sensitivity, especially in producing highly multiply charged ions from proteins such as cytochrome C and myoglobin, which require only low picomole quantities injected on column. A gradient separation of several peptides and proteins was achieved. A gradient from 100% aqueous (2% acetic acid) to 50/50 H₂O/ACN (2% acetic acid) was run using a 320 micron i.d. x 30 cm long packed silica column and a gas-assisted electrospray probe (2), which improved spray stability. Solvent delivery was performed by conventional SSI reciprocating pumps operating at 0.6 mL/min, with a pre-injector split to 10 uL/min passing through the column. Full scan spectra at 7 secs/scan were obtained from 6 picomole of myoglobin and cytochrome C injected (3uL) on column.

**REFERENCES**

CZE and Nanoscale Capillary LC Coupled with Electrospray ionization Mass Spectrometry for the Analysis of Proteins

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Electrospray ionization (ESI) has found two major roles in mass spectrometry. The ability of ESI to produce multiply-charged gas phase ions from labile analytes of high molecular weight, such as proteins, has attracted much attention. Less well appreciated is the facile manner in which ESI can be used for coupling mass spectrometry with liquid phase separation systems, including capillary zone electrophoresis (CZE) and liquid chromatography. The work here reported has used both features of ESI: the analysis of proteins via on-line separations with mass spectrometry.

An electrospray ionization interface (Analytica of Branford) has been used with a single quadrupole mass spectrometer (Nermag R1010ES). This system has been coupled with both capillary zone electrophoresis and nanoscale capillary LC systems fabricated in-house. The performance of the combined systems was evaluated via analysis of mixtures of different forms of cytochrome-C and myoglobin. These mixtures were analyzed using full scan data acquisition (m/z 500-2000), at the 2 to 4 pmole level (Figures 1 and 2).

The principle application of this research has been the characterization of the class of metabolic enzymes known as glutathione-S-transferase (GST), which detoxify electrophilic alkylating xenobiotics (mutagens, carcinogens, and other noxious chemicals). The presence of a variety of different isozymes of GST, each with differential, overlapping substrate specificity, allows aerobic organisms to detoxify a wide variety of reactive xenobiotics. The type and amount of the different isozymes of GST present in a given tissue (i.e. the isozyme pattern) will determine the sensitivity of the tissue towards the toxic effects of electrophilic compounds.

Nanoscale separation systems coupled with ESI/MS have proven to be capable of characterizing the GST isozyme pattern in tissue samples. Constant infusion of a crude mixture of GSTs from rabbit liver into the ESI/MS system gave a mass spectrum (Figure 3) containing two major components, identified as the alpha-1 (MW=25,650) and alpha-2 (MW=25,350) isozymes, along with at least four minor components (MWs from 24,000 to 26,000). Analysis of this mixture by CZE/ESI/MS resulted in the separation of the alpha-1 and alpha-2 isozymes. Application of nanoscale capillary LC/ESI/MS methodology to this rabbit liver GST sample gave seven distinct chromatographic peaks, all corresponding to GST isozymes with molecular weights between 25,000 and 26,000.
Electrospray ionization / Mass Spectrometry

Glutathione-S-Transferase (rabbit liver)


Std. Dev. = 0.34  Std. Dev. = 3.63  Std. Dev. = 8.88  Std. Dev. = 18.62  Std. Dev. = 14.17

COV (%) = 0.210  COV (%) = 0.0102  COV (%) = 0.0341  COV (%) = 0.0745  COV (%) = 0.0562

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HIGH-RESOLUTION MASS ANALYSIS AND ELECTROSPRAY IONIZATION

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An electrospray ionization (ESI) source has been coupled to a magnetic sector mass spectrometer operated at acceleration potentials of 3 kV to 7kV. Analyses have been performed that demonstrate the determination of mass, charge state, and stable isotope distribution from high resolution mass analysis of multiply charged ions. Linked scan (constant B/E) analyses of the [MH]+, [M+2H]2+, [M+3H]3+, and [M+4H]4+ species have been obtained for several peptides to determine the feasibility of performing MS/MS experiments on the multiply charged ion species.

The ion acceleration region of the mass spectrometer consists of four lens elements arranged as a capillary (C), skimmer 1 (S1), and conical lens. The acceleration potential of the mass spectrometer is set by the potential of the S1 element. We have observed that the magnitude of the voltage difference between skimmer 1 and the capillary where the ions are entering the acceleration region can affect both the charge-state distribution of the ions and the extent of fragmentation. For example, when a capillary/skimmer potential of 10 V is used, the ESI mass spectrum of a polyethylene glycol (average M.W. = 2000) shows a mixture of predominantly doubly and triply charged species. If the capillary/skimmer potential difference is increased to > 300 V, the mass spectrum show only the singly charged species ([M+Na]+). In other experiments with the peptide bradykinin, it was observed that raising the capillary/skimmer potential difference causes extensive fragmentation. The fragment ions observed are consistent with a low-energy CID process.

Linked-scan (constant B/E) analysis can be performed for ions dissociating on the first field-free region of the mass spectrometer. This provides higher collision energies than obtained for the "in-source" (capillary/skimmer region) dissociation. Dissociation of the [M+2]2+ species from bradykinin shows characteristic side-chain fragmentations that are characteristic of high-energy CID processes. These fragment ions were not observed in the "in-source" fragmentation.

High-resolution electrospray ionization mass spectra indicate that it is possible to determine the relative isotope distribution for the molecular ion species with greater accuracy than FAB, due to the absence of a matrix compound in the electrospray ionization process. The reduction in chemical background provides better resolution for ESI-MS compared to FAB-MS and avoids chemical reactions with the FAB matrix (oxidation/reduction) that prevent the reliable determination of isotope distributions. ESI-MS shows the natural charge state for a cyclodextrin dimer that has a doubly charged cation, while the FAB mass spectrum shows a reduction to a singly charged species.
"Normal" ESI mass spectrum of poly(propylene glycol) Average M.W. ~2000u

Mass spectrum for a capillary/skimmer potential difference of 400 volts.
Electrospray ionization has proven phenomenally useful in the analysis of thermally labile polar molecules, especially for peptides and proteins. The development of this atmospheric ionization technique initially began on quadrupole instruments because of the lower voltages required in operation. Interfacing electrospray to high-voltage magnetic sector instruments requires reducing the pressure from atmospheric pressure (ionization) to high vacuum (ion acceleration) in a step-wise fashion in order to prevent corona discharges. We developed a simple single staged electrospray source based on the Fenn and Whitehouse design for a VG ZAB-E, in order to take advantage of the better mass accuracy, higher resolution and extended mass range of a high performance mass spectrometer. Having a similar electrospray interface for a Finnigan TSQ700, we can make a direct comparison of electrospray on a quadrupole and a magnetic sector mass spectrometer. Interfacing electrospray to the different instruments has relative merits. Parameters to consider include: mass range, sensitivity, resolution, mass accuracy, complexity of the source, and ease of operation.

- Mass Range - most biomolecules we have studied result in a distribution of multiply charged ions below 2000 m/z with a few biopolymers showing distributions up to 3500 m/z. The larger mass range is not necessarily a requirement for success in electrospray.

- Sensitivity - electrospray interfaced to a quadrupole or a magnetic sector instrument exhibits, for the most part, similar sensitivities. Array detection could be employed on the magnetic sector instrument which would increase the detection limits.

- Resolution - resolutions of 10000 have been observed with electrospray on a magnetic sector instrument. Higher resolution aids in mixture analysis. Shown below is the theoretical resolution for bovine insulin (mw 5733.5), lysozyme (mw 14305), and carbonic anhydrase (mw 29000). Included with each is a 25% impurity of the oxidized molecule. On the +5 ion for insulin, the resolution narrows the peak making the determination of the average mass more reasonable. For molecules in the 5000 mw range a resolution of 1000 should be sufficient. The lysozyme molecule shows that 300 and 1000 resolution is inadequate and shifts the mass measurement to higher mass because of the contribution of the oxidized species. For this mw range, a resolution of 2000 is adequate. Similarly for the +20 ion for carbonic anhydrase, the resolution of 5000 would result in reasonable mass accuracy.

- Mass Accuracy - Mass accuracy of 0.01% is achievable on the quadrupole which is equivalent to what is obtained on the magnetic sector instrument. However, it has been shown that 0.003% mass accuracy to 17000 u is possible on a magnetic sector instrument.

- Source Complexity and Ease of Operation - Pressure and source contamination are critical in the electrospray source and appear to have a more dramatic effect on the magnetic sector instrument.

Effect of Resolution on Bovine Insulin (+5) Ion

Effect of Resolution on Lysozyme (+10) Ion

Effect of Resolution on Carbonic Anhydrase (+20) Ion
An Electrospray Ionization Time-of-Flight mass spectrometer (ESPI-TOF-MS) has been designed and tested for the rapid analysis of biological compounds. The Electrospray Ion Source and the TOF mass filter together comprise an extremely powerful chemical analysis tool. The unlimited mass range of the TOF-MS allows detection of macromolecules beyond the reach of dispersive instruments. In addition, simultaneous mass detection and rapid scan speed make it an ideal ion detector for rapid chromatographic separation techniques such as microbore LC or CZE. The inherent simplicity in operating such an instrument may also appeal to biomedical researchers outside of mass spectrometry.

The application of several variants of ESPI-TOF-MS as they relate to the detection of biologically significant compounds is considered, with an emphasis placed upon maximizing the normally low duty cycle associated with "chopping" a CW ion beam. Extensive studies have been performed to determine the best method(s) to produce ion packets suitable for TOF mass filtering. In all cases, the goal was to maximize duty cycle while maintaining a marginally acceptable mass resolving power.

One method has been shown to be far superior in terms of enhancing the sensitivity of the ESPI-TOF instrument. With the appropriate selection of static and pulsed electric fields, a DC beam of Electrospray Ions has been stored and modulated to produce TOF spectra for compounds ranging from 100 - 1000 m/z. Ion sensitivity has been measured to be significantly greater than for traditional rastering or chopping approaches. Compromises in ion energy spread are minimized, and can be treated by further energy compensation.

Figure 1. Schematic of the Electrospray-TOF-MS
Figure 2. Spectrum of Arginine (MW = 174.2 amu) in 50:50 Methanol/Water. Sample concentration is 0.1 grams per liter.
DEVELOPMENT OF AN ELECTROSTATIC ION INJECTION DEVICE FOR FOURIER TRANSFORM ION CYCLOTRON RESONANCE STUDIES OF ELECTROSPRAYED IONS

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This group has recently dedicated considerable effort to the development of a simple electro spray ionization (ESI) interface for use with Fourier transform ion cyclotron resonance (FT-ICR) detection. This source incorporates the Fenn/Whitehouse type glass capillary ion transfer tube in a two-stage pressure reduction, capacitance -type molecular beam source. In this configuration, ion injection into the magnetic field and ion cell would be accomplished by direct injection oriented along the magnet's central axis in a manner analogous to that employed by Smalley et al.1. Studies in the past year emerged along two paths, i) determining the minimum parameters required for operation of the ESI-FT-ICR interface and ii) developing this “minimum interface” using a quadrupole mass spectrometer in an effort to further define the essential functional requirements of the interface.

In order to understand charged particle injection into the FT-ICR cell, an electron injection source was constructed as shown in Figure 1 and mounted to the right side of the FT-ICR vacuum chamber (see Figure 2). The motivation for injecting electrons from a distal source was threefold. First, the magnetic chaos at the end of the magnetic could be probed directly using a very light particle that cannot penetrate fields which are non-conducive to injection—especially at low energies. Secondly, this electron “test bench” would allow both visual and magnetic alignment of the ESI interface. Finally, the effect of vacuum chamber pressure could be crudely probed for the electrons traversing the chamber. After physical alignment of the source, it was found that electrons exiting the skimmer passed through the cell and struck the collector plate nearly a meter away without any detectable signal (using a picameter) striking the cell plates. This was found to be true even down to 5 eV electrons. Argon admitted to the right side of the FT-ICR vacuum system (as depicted in Figure 2) attenuated the electron signal detected at the collector plate by nearly 20% on going from 1 x 10⁻⁴ torr to 1 x 10⁻¹⁰ torr argon pressure.

Without altering the physical alignment of the vacuum system, a simple ESI interface was assembled and installed on the FT-ICR vacuum system depicted in Figure 2. A 300 µ borosilicate glass capillary having both ends vapor plated with gold was fitted to the standard 2 ½ conflat vacuum cross so that the exit from the capillary was held rigidly a few millimeters from the entrance to a 460 µ copper skimmer. The capillary skimmer region of the interface was pumped by a mechanical pump capable of maintaining approximately 0.300 torr. The exit from the skimmer was fitted with a large, three element tube lens. Pumping after the skimmer was provided by the right side of the differential FTMS-20GG vacuum system giving a pressure of nearly 1 x 10⁻⁴ torr in the right differentially pumped region and approximately 1 x 10⁻³ torr in the left. A 0.001 M NH₄⁺(OAc⁻) solution was electrosprayed into the system (positive ion mode) giving a steady 1 pA positive current detectable at the conductance limit plate at the center of the ion cell. A large current (approximately 0.6 pA) was detected at the right cell plate and no current (< 0.1 pA) was detected on the left cell plate or collector plate indicating that the ions were very defocused. No ions could be detected by FT-ICR in either cell region.

The ESI interface above was mounted onto an Extrel quadrupole mass spectrometer with the skimmer exit region pumped by a 700 IS⁺ cryopump. Ion transmission through the skimmer was found to improve with better pumping conductance behind the skimmer. This conductance was improved by decreasing exit dead volume and refining the larger, more restrictive tube lens with a more open tube lens/Elzing lens design. The capillary was placed in an X-Y tilt adjustable mount for more controllable placement of the capillary exit. Pumping in front of the skimmer was improved by placing the skimmer closer to the throat of the tee in which it was mounted. Initial studies using the quadrupole test system indicate that aggressive drying in the nozzle/capillary entrance region is required for transmission of ions. Further, preliminary data indicated that the width of the energy spread for ions emerging from the source is directly related to the amount of drying before entering the capillary. Various configurations of gas flow guides have been employed to bathe this region with warm N₂. It was found that more directed gas flow yielded improved performance.


2. Research sponsored jointly by the Office of Health and Environmental Research, U.S. DOE under contract DE-AC05-84OR21400 with Martin Marietta, Inc., and the Postgraduate Research Program under DE-AC05-76OR00033 between the U.S. DOE and Oak Ridge Associated Universities.

Figure 1. Electron source used to mimic ion injection through a skimmer placed outside the magnetic field.

Figure 2. One of the simple test ESI-FT-ICR interfaces evaluated in this work.
Advanced Methods for Structural Characterization of Large Multiply Charged Ions: Obtaining Charge State Determination and Improved Sensitivity

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There is growing interest in obtaining structural information for large molecules by the methods of tandem mass spectrometry. Electrospray ionization (ESI) now allows the efficient production of high molecular weight ions having high charge states. The application of existing instrumental methods for this purpose encounters several problems. One is that the primary ion beam flux is, to a crude approximation, inversely related to the square of molecular weight for large molecules due to the number of charges on each ion, the width of the charge state distribution, and the limitations upon electrospray ion currents. Since the number of "useful" and desired fragmentation pathways increase approximately linearly with molecular weight, the sensitivity demands increase approximately with the inverse third power of molecular weight. A second problem for methods utilizing multiply charged parent ion is the necessity of determining the charge states of product ions from tandem experiments. The combination of these problems will grow dramatically with molecular weight, currently limits the application to high molecular weight biomolecules.

A solution to these problems, which we plan to investigate, is based upon the use of coincidence detection methods, an approach which exploits the fact that molecular ions for higher molecular weight compounds produced by ESI, and more importantly, their collision induced dissociation (CID) products will often be multiply charged.

A CID mass spectrum represents a summation of signals resulting from different dissociation processes occurring for molecular ions, each pathway characterized by different statistically determined (e.g., RRKM) frequency of occurrence. The basis of our approach will be to determine the product ions arising from CID of single parent ions. The number of different CID processes possible for a large polypeptide is very large. Complex dissociation processes can occur (particularly at higher collision energies), including side chain losses, sequential dissociation processes, and perhaps charge or proton transfer to the collision gas. The additional complications arising due to the range of possible product ion charge states arising from dissociation of a multiply charged parent ions adds an element of ambiguity that for large molecules generally precludes spectral interpretation unless the structure is already largely established.

Assume a molecular ion of mass M and charge Z. Both M and Z are known from interpretation of the ESI mass spectrum. Of interest is the situation where molecular ion internal energy does not greatly exceed that required for dissociation on the mass spectrometric time scale, so that more extensive dissociation processes are avoided. Here the most likely dissociation processes can be generalized by reactions [1]-[7].

\[
\begin{align*}
M^Z & \rightarrow m_a^x + m_b^y + m_c^z + m_d \\
M^Z & \rightarrow m_a^x + m_b \\
M^Z & \rightarrow m_a^x + m_b y^+ \\
M^Z & \rightarrow m_a^x + m_b y^+ + m_c \\
M^Z & \rightarrow m_a^x + m_b y^+ + nV + z^+ \\
M^Z & \rightarrow m_a^x + m_b y^+ + m_c z^+ + m_d
\end{align*}
\]

where \( m_a + m_b + m_c = M \), \( x + y + z = Z \), and \( y^+ \) and \( z^+ \) designate (undetected) charge loss. Each reaction may represent hundreds, or even thousands, of possible CID processes. It should also be noted that the individual neutral products (\( m_b \), \( m_c \), or \( m_d \)) may represent the sum of several smaller indicated neutral species. The vast majority of all CID processes of interest are expected to conform to these general reactions, unless excessive internal energy is deposited in the molecular ion (facilitating additional sequential dissociation steps).
An important feature of most CID processes for each multiply charged parent ions is the formation of more than one product ion. With the possible exception of simple cleavage to form "complementary ions" by reaction [3], the absence of charge state information prevents reliable assignment of peaks in the mass spectrum to specific CID processes. If product ions arising from individual CID events can be correlated, our evaluation has shown that interpretation and unambiguous charge state assignment becomes feasible in most cases. This is possible for two reasons: the fact that total mass and charge (of the parent) are known, and that ion charge is restricted to integral values.

The correlated detection of dissociation products can potentially yield enormous gains in sensitivity. Since detection is accomplished for single events, conventional problems related to resolution would rarely be significant; rather, temporal resolution is of significance along with m/z measurement accuracy. Since events at much less than one per second should be interpretable with confidence, sensitivity gains can be substantial. In addition, the problem of "spectral congestion", noted in our studies of large molecule CID² should largely be moot; i.e., different species having the same nominal m/z (but perhaps different charge) would be temporally resolved. Clearly this approach would yield an abundance of mechanistic information, otherwise quite difficult to obtain.

The coincidence detection approach could be implemented in several ways. One approach could involve a tandem instrument with a TOF second stage. At low ion flux (readily obtained for ESI), and with m/z selection in the first stage, each TOF spectrum would be limited to dissociation of only a few or (ideally) only one parent ion. Thus, TOF detection in this limit inherently provides coincidence detection, but at a substantial cost in terms of sensitivity (at least for conventional TOF methodologies) due to the low duty cycle. A much more powerful alternative might involve tandem double focusing sector instrumentation with an array detector. Such a detector must have both a wide m/z range for simultaneous detection, and provide for adequate temporal resolution to allow product correlation. We plan to investigate such correlated detection methods, as well as the ancillary issues related to data analysis and interpretation.

References


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Peptide Fragmentation in $^{252}$Cf-PDMS

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Recent investigations have indicated that the prompt, metastable fragmentation of peptides observed in $^{252}$Cf-PDMS provides sufficient information for effective peptide sequence analysis. Compared to other mass spectrometric methods used in peptide sequencing, the fragmentation of peptides observed in PDMS is unique, both in terms of the time scale of the fragmentation, and the amount of energy deposited in the parent molecule, inducing fragmentation. Therefore, we began a study of the fragmentation of peptides in PDMS, to characterize the factors that influence fragmentation, and to compare this fragmentation to that observed in other mass spectrometric methods.

Our initial examination of the fragmentation of peptides in PDMS used the nonapeptide bradykinin (RPPGFSPFR) as a model compound. The PD mass spectrum of bradykinin in Figure 1 was obtained by adsorbing approximately 250 pmol of the peptide onto a nitrocellulose matrix. The types of fragment ions observed in Figure 1 are very similar to those observed in MS/MS under high energy CID conditions: extensive $a_n$, $b_n$, $c_n+2$, $x_n$, $y_n+2$, and $z_n+1$, fragment ions as well as the higher energy $d_n$, $v_n$, and $w_n$ side-chain fragment ions.

To determine whether a charge-remote or a charge-induced fragmentation mechanism is taking place in the fragmentation of peptides by PDMS, analogs of bradykinin in which both the N- and C-terminal basic arginine residues were removed were analyzed. In the PD mass spectrum of des-arg$^9$ bradykinin, no C-terminal fragment ions were observed. This indicates that charge-remote fragmentation may be occurring because removal of the basic C-terminal arginine eliminated all C-terminal fragment ions. The PD mass spectrum of des-arg$^1$ bradykinin showed C-terminal fragment ion comparable to those seen in the spectrum of bradykinin, but also weak N-terminal fragment ions. The observation of N-terminal fragment ions in the spectrum of des-arg$^1$ bradykinin does not preclude a charge-remote fragmentation mechanism because the des-arg$^1$ bradykinin molecule still include a possible site for N-terminal protonation: the α-amino group of the N-terminal proline. When this N-terminal α-amino group was acetylated, the N-terminal fragment ions were
not observed in the PD mass spectrum, while the C-terminal fragment ions remained unaffected. Our interpretation of these results is that charge-remote fragmentation is prevalent in the PD mass spectra of peptides.

To examine the effect of the nature of the charge site on the fragmentation, we examined derivatives of bradykinin in which the N-terminal basic amino acid was altered. Figure 2 shows the results of this study. In this figure the relative intensities of N-terminal a_n series of fragment ions is plotted for four peptides with varying N-terminal charge-sites. The N-terminal triphenylphosphonium (TPP) derivative of bradykinin, with a fixed positive charge on the N-terminus yielded the most intense N-terminal fragment ions. The relative intensities of the N-terminal fragment ions of the arg^1, his^1, and lys^1 bradykinin analogs follow the same trend as the relative gas-phase basicities (1) of these amino acids: arg > his > lys.

The fragmentation observed in PDMS is not limited to small peptides. As Figure 3 shows, fragmentation of an amino acid chain is also observed in the PD mass spectra of small, native proteins such as ribonuclease A (M_r = 13,682.4)

![Figure 2](image)

**Figure 2.** Relative intensity of the N-terminal a_n series of fragment ions for four bradykinin analogs.

![Figure 3](image)

**Figure 3.** Fragment ion observed in the PD mass spectrum of ribonuclease A (M_r = 13,682).

LINKAGE POSITION DETERMINATION IN LITHIUM CATIONIZED DISACCHARIDES: TANDEM MASS SPECTROMETRY AND SEMIEMPIRICAL CALCULATIONS

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Fast atom bombardment ionization (FAB) in combination with tandem mass spectrometry of lithiated disaccharides is used to differentiate the linkage position of five isomeric sugars as well as investigating the gas phase mechanisms of various ion formation. In this study we show how the linkage position of different isomeric disaccharides can be distinguished when the monolithiated precursor undergoes collision induced dissociation (CID) after FAB ionization (Table 1). Deuterium and 18O labeling studies were used in combination with product and precursor ion scans which showed that reducing ring opening occurs followed by two-, three-, and four-carbon chain neutral losses via a retro-aldol rearrangement (Scheme 1). Carboxyl migration via keto-enol tautomerization is postulated as an isomerization process that occurs during CID, thus enabling specific cleavages for each different isomer. Semiempirical calculations of both the hemiacetal and hydroxy aldehyde forms support experimental data which indicates that the lithium ion is penta-coordinate between the two rings with the most stable structure giving a heat of formation of -406 kcal/mole (Figure 1).

Table I. Tabulated Product Ion Spectra of Different Linked Disaccharides

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linkage</th>
<th>m/z</th>
<th>Neutral</th>
</tr>
</thead>
<tbody>
<tr>
<td>gentiobiose</td>
<td>β-1,6</td>
<td>331</td>
<td>X</td>
</tr>
<tr>
<td>melibiose</td>
<td>α-1,6</td>
<td>289</td>
<td>X</td>
</tr>
<tr>
<td>maltose</td>
<td>β-1,4</td>
<td>259</td>
<td>X</td>
</tr>
<tr>
<td>lactose</td>
<td>α-1,4</td>
<td>229</td>
<td>X</td>
</tr>
<tr>
<td>laminarbiose</td>
<td>β-1,3</td>
<td>187</td>
<td>X</td>
</tr>
<tr>
<td>nigerose</td>
<td>α-1,3</td>
<td>169</td>
<td>X</td>
</tr>
<tr>
<td>sophorose</td>
<td>α-1,2</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>trehalose</td>
<td>α-1,1</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>
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Scheme 1

Figure 1

-406 kcal
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GAS PHASE PROTON AFFINITY OF RIBONUCLEOSIDES BY FAST ATOM BOMBARDMENT MS/MS

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The evaluation of the proton affinity (PA) of nucleic acid constituents in vacuo, provides data unaffected by the solvent effect thus enabling straightforward structural function correlations. The determination of the pK values of nucleotides has been widely investigated both theoretically and experimentally for its implication in the base-base recognition processes (1). The gas phase chemistry of the all set of proton bound heterodimers containing the four DNA nucleosides has provided information concerning the relative PA of the examined molecules in an hydrophobic environment (2). A recently proposed fab-bracketing approach (3) allowed the determination of their absolute PA values by applying the eq. 1, Where the unknown value of the nucleic acid component

\[ \text{PA}_N = \frac{\ln (k_{N/k_A1}) \cdot \text{PA}_{a2} - \ln (k_{N/k_A2}) \cdot \text{PA}_{a1}}{\ln (k_{N/k_A1}) - \ln (k_{N/k_A2})} \]  

(\text{PA}_N) can be determined from the known values of two reference amines (\text{PA}_{a2}, \text{PA}_{a1}) by means of the experimentally determined rate constants (k) for the unimolecular dissociations of a given proton-bound heterodimer. This method is sensitive to very small variation in the basicity properties of the examined molecules and has been therefore applied in the evaluation of the substituent effect on the absolute PA of nucleosides.

The PA's of ribonucleosides (N) and deoxyuridine (dU) have been determined as described above, using triethylamine and tripropylamine as reference compounds for G, A and C and pyrrolidine and piperidine for U and dU. The PA's of ribonucleosides (N) differ from those of the correspondent deoxyribonucleosides (dN) by 0.6-1.0 kcal/mole (table). This effect has to be associated to the lack or presence of an OH group in the 2' position of the sugar portion of the nucleoside. The lower basicity of ribonucleosides with respect to the deoxy analogs, already verified in solution (1), is associated to the electron withdrawing effect of the extra hydroxyl group which provides them an higher stability towards depurination. A deeper insight into the physical meaning of the observed PA differences (0.6-1.0 Kcal/mole) can be obtained from the unimolecular dissociations of the proton-bound dimers formed by ribo and deoxyribo nucleosides containing the same aglycone moiety. In the figure are reported the MIKE spectra of the species [dC+C]+ and [dG+G]+. The partitioning of the proton among the two interacting nucleosides always favor the formation of (dN+H)+ ions. Moreover the relative intensity of the daughter ions shows that the PA difference of the guanine containing nucleosides is lower than that of the cytosine analogs. The data reported in the table allow also to conclude that the relative basicity of the unmodified gaseous ribonucleosides follows the same order of the correspondent deoxy-analogs (3), i.e. G > A > C > U. The reliability of the proposed method is further evidenced by the results obtained with the pyrimidine containing nucleosides. The measured
proton affinities of deoxythymidine (dT) and uridine (U) differ from that of deoxyuridine (dU) by 0.6 and 1.0 kcal/mole, respectively.

It is possible, therefore, to evaluate selectively both the contribution of the methyl group in position 5 of the base and that of the hydroxyl function in position 2' of the sugar moiety.

The enhanced basicity of dT with respect to dU is due to the electron releasing effect exerted by the alkyl group adjacent to the amide function of the pyrimidine base. This result suggests, also, that the O4 represents the protonation site of both nucleosides in agreement with the chemistry of the same species in the condensed phase (1). The PA difference between dU and U is due to the electron withdrawing effect of the 2'-OH group and falls in the same range previously discussed for the purine nucleosides. The overall basicity enhancement of dT with respect to U (ΔPA=1.6 kcal/mole) has to be ascribed to the presence of the 5-CH3 group and to the lack of the 2'-OH function in the deoxythymidine molecule.

In conclusion the kinetic approach applied in the determination of the proton affinities of nucleosides enables the evaluation of PA differences of less than 1 Kcal/mole which can be correlated to the structure of the examined molecules.

References
1 Saenger, W. Principles of Nucleic Acid Structure; Springer-Verlag: New York, 1984; pp. 105-115
OBJECTIVES. The dimeric indole alkaloids vincristine, VCR, vinblastine, VBL, and vindesine, VDS, are used in the chemotherapy of several neoplastic diseases. In Fig. 1, the substitutes, in the vindoline part, for VCR, VBL, and VDS, respectively are: \( R_1 \) COOCH\(_3\), COOC\(_3\)H, CONH\(_2\); \( R_2 \) COOCH\(_2\)CH\(_3\), OCOCH\(_3\), OCOCH\(_3\); OH; and \( R_3 \) CHO, CH\(_3\), CH\(_3\). The catharantine part is the same for all three compounds. Our objectives were to compare the collisional activation decomposition of these compounds using FAB and to select strategic ions for the subsequent search for metabolites in urine, serum and tissues.

METHODOLOGY. Matrix: glycerol. The VG Trio-3 triple quadrupole mass spectrometer was operated in static FAB mode using xenon at 8 kV and 1.2 mA. CAD conditions: nitrogen at 10\(^{-5}\) X 10\(^{-6}\) mtorr, 8-18 eV. Daughter spectra were obtained from 30 ions and parent spectra from 39 ions selected from various daughter ion spectra.

RESULTS AND DISCUSSION. We divided the FAB spectrum of VCR (Fig. 2) into two parts. The substituent-dependent ions, appearing at their respective masses, were almost identical for VCR and VBL, while vindesine yielded the same ions but usually of much lower intensities. The \((M + H)^+\) ions of VCR AND VBL are base peaks; VDS is intense (15%), but its base peak is at m/z=154. The other substituent-dependent ions, including the loss of water, methanol, the \( R_1 \) substituents and the loss of the catharantine moiety are present in all three vincas at approximately the same intensities (1-8%). In the substituent-independent group (m/z<500), the three compounds behave nearly identically, with intense (23-52%) peaks at m/z=144, 140, and 122, and a structurally and diagnostically interesting ion at m/z=355 (see later). The m/z=154 ion is intense in both VCR and VBL and is the base peak of VDS.

In the daughter spectrum of \((M + H)^+\) (Fig. 3) the substituent-independent ions, e.g., m/z 705 and 765, appear, as expected on the basis of energetic considerations, at higher intensities than in the straight FAB spectra. The daughter spectra of VBL and VDS show similar decomposition products, losing the \( R \) substituents. In Fig. 4 portions of the daughter ion spectra of the \((M + H)^+\) ion of VCR, the intense substituent-dependent ion at m/z=765, and an ion, selected as the vindoline marker, at m/z=555. The spectra are aligned to reveal a series of identical losses from the parent ions. The CAD processes A, B, C, etc., are the same for all three parents within the \( R \)-containing vindoline part of the molecules and correspond to the loss of \( \text{H}_2\text{O}, \text{C}_2\text{H}_4\text{O}_2, \text{C}_2\text{H}_2\text{O}_2, (\text{C}_2\text{H}_4\text{O}_2+\text{H}_2\text{O}), (\text{C}_2\text{H}_4\text{O}_2+\text{CH}_3\text{OH}), \text{C}_2\text{H}_2\text{O}_2+\text{C}_2\text{H}_2\text{O}_2, \) and 2 \( \text{X} \text{C}_2\text{H}_4\text{O}_2 \), respectively. It is noted that processes D, E, F, and G assume two-step decompositions.

Although of low intensity, m/z=555 is rich in information and is representative of the catharantine ring. Fig 5b shows the parent spectrum confirming that this ion directly originates from the molecular ion of VCR. It is noted here that parent ion spectra were obtained for all ions in the various daughter spectra to confirm their origins. Fig. 5a shows the daughter ion spectrum of the m/z=555 ion. The higher mass region of the spectrum has already been discussed (Fig.3). The ions with lower masses can also be derived from the structure of m/z=555 (Fig. 6). Ions at m/z 285, 122, and 108 also appear in the straight FAB spectrum of VCR, in fact, at rather higher intensities (Fig. 2).

Fig. 7 shows similar information for the m/z=353 ion. Fig 7b shows two parents. One is the molecular ion of VCR, the other is an original small impurity, one that turned out to be a vincain analog with an \( H \) instead of \( \text{CHO} \) on the 1-N in the vindoline ring. The m/z=355 ion can be used to explain the appearance of the m/z=144 and 224 ions in the daughter ion spectrum of vincristine and the m/z=130 ion appearing in straight FAB. In addition, the structure of the m/z=353 ions leads to the formation of a few ions, e.g., m/z 293 and 210, which are not directly relevant to our objectives. To complete the explanation of all important ions both in the straight FAB and daughter spectra of VCR, the structures of three additional ions, at m/z=140, 154 and 168, are shown in Fig. 1. None of these is substitute-dependent and all are of high abundance, in fact, the m/z=154 peak is the base peak for VDS.

Strategy for metabolite identification. If the m/z=555 ion is present, there are no metabolic changes involving R and 18 carbon substitutes. If m/z=353 is found, the metabolic process involved only the vindoline parts which contains the R substitutes. If m/z=355 is not found, the presence of m/z=140, 154, and 166 indicates metabolic changes on substituents on 16 or 18 carbons. If only m/z=130 and 144 are present, metabolic changes occurred on substituents on 4 carbon. To search for the \((M + H)^+\) ions of potential metabolites, find all parents of the markers and, in turn, look for the grandparents, etc. Finally, the interpretation of the ions in the daughter spectra of the ions thus found will help to confirm the identification of the metabolite(s).

CONCLUSIONS. (a) Apart from intense \((M + H)^+\) ions of VCR, VBL, and VDS, the substituent-dependent fragments were low, but measurable, intensity; (b) In the daughter ion mode, several substituent-independent ions were present in considerable intensities; (c) Representative ions were selected for the vindoline and catharantine portions; (d) Several selected ions will, in the parent ion mode, help to detect and identify metabolites in biological materials.

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MULTIPLE STAGE MASS SPECTROMETRY IN THE STRUCTURAL CHARACTERIZATION OF ORGANOPHOSPHORUS COMPOUNDS

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Electron ionization of organophosphorus compounds often yields a number of small fragment ions, which complicates the interpretation of unknown compounds of this type. By performing sequential collision-induced dissociation, or multistage mass spectrometry, on the molecular ion and subsequent fragment ions, the fragmentation pattern can be simplified and information about the structure of the molecular ion may be obtained. Further information can be obtained by reacting the fragment ions with neutral molecules in ion-molecule reactions. In this work, we describe the use of multistage mass spectrometry combined with energy resolved CAD or hydrogen deuterium exchange in investigations of the structures of fragment ions common to many organophosphorus cations.

The fragment ion C₂H₄O₃P⁺ (m/z 109) is common to many organophosphorus esters. Four stable isomers of m/z 109 were generated from the dissociation of trimethyl phosphate, triethyl phosphate, trisopropyl phosphite and ethylene phosphonate. Upon CAD, each of these isomeric ions of m/z 109 fragment differently to give an energy resolved plot that is characteristic of that isomer (Figure 1). In Figure 2, the collisionally induced dissociation spectrum of m/z 109 obtained by CAD of the molecular ions of dimethyl chlorophenyl thiophosphate is compared to the CAD spectrum of the reference ion generated from trimethyl phosphate. The four isomer isomeric ions of m/z 109 can also be distinguished on the basis of the number of acidic protons they contain through the use of hydrogen deuterium exchange reactions: the dimethyl isomer does not undergo H/D exchange with deuterated ammonia, acetic acid or water, the ethyl isomer exchanges one hydrogen atom with deuterium, the cyclic ester exchanges two hydrogens and the vinyl isomer three.

Multistage mass spectrometry was also used to study the structures of other fragment ions from the molecular ions of organophosphorus compounds. By adjusting the conditions of CAD of the molecular ion of dimethyl chlorophenyl thiophosphate it is possible to fragment the molecular ion into only a few ions, including m/z 109 and 125. The structure of the fragment ion of m/z 125 produced from aromatic thioesters has been proposed to be that of the sulfur analog of the dimethyl isomer of m/z 109 (see figure 3). However, the CAD spectrum of the m/z 125 fragment ion produced from dimethyl chlorophenyl thiophosphate is markedly different from that of the sulfur analog and is virtually identical to that of the fragment ion of m/z 125 from 4-chlorotoluene (Figure 3). We conclude that the structure of the m/z 125 fragment ion of these pesticides contains the aromatic moiety rather than the heteroatom moiety. The observation of a homologous fragment of m/z 139 from the molecular ion of diethyl chlorophenyl thiophosphate under similar CAD conditions indicates that transfer of C₂H₄ to the aromatic portion of the molecule of the ethyl esters occurs. Compounds of similar structure but without the chlorine substituent also exhibit alkylene transfer to the aromatic ring portion of the ion. CAD of pesticide analogs ionized by protonation also produces product ions that, upon CAD, yield structurally characteristic information about the neutral molecules in a similar manner as described here for the molecular ions. Information obtained from CAD of the molecular ion and the fragments of the molecular ions of organophosphorus compounds can be used to characterize the structures of these compounds.
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Fig. 1

Fig. 2

a) CAD of m/z 109 from

CH₃O-P-O-CH₃

81 91

b) CH₃CH₂O-P-OH

81 91

Fig. 3

a) m/z 125 from

S²⁺ CH=CH² O-P-O-CH₃

89

b) CH₃O-P-O-CH₃

89

c) 79

1188
The dehydrobenzenes (o-, m-, and p-benzynes), 1 - 3, constitute a fascinating class of reactive organic intermediates that has inspired numerous experimental and theoretical studies. Ortho-benzyne has been extensively studied over the last forty years to establish its geometric and electronic structures, its chemical reactivity, and its thermodynamic properties. Recently, derivatives of para-benzyne have attracted considerable attention as potential bio-active intermediates in the DNA-cleaving action of calichemicins and esperimicins. A related system, the α,n-dehydrotoluenes (n = 2, 3, 4), 4 -6, have not been studied as intensively as the benzynes. α,3-Dehydrotoluene, however, has been proposed to be essential in the activity of the DNA cleaving necarzinostatin chromophore. Therefore, we have been studying the thermochemistry of the dehydrotoluenes and the benzynes in a flowing afterglow-triple quadrupole instrument and report here the absolute heats of formation of ortho-, meta-, and para-benzyne and α,2-, α,3-, and α,4-dehydrotoluene as derived from energy resolved collision-induced dissociation (CID) measurements.

We have recently demonstrated how collision-induced α-elimination reactions of halocarbonanions can be used to obtain carbene thermochemistry in a flowing afterglow-triple quadrupole instrument. We have now extended this approach to more remote (α,ω) eliminations to obtain thermochemistry for unsaturated compounds and diradicals. For the benzynes the procedure involves regiospecific generation of the chloropheny! anions in the gas phase followed by measurement of the activation energies for chloride loss upon collision activation. The measured activation energies can be combined with the heats of formation for CI' and the precursor chloroanions to give the heats of formation for the neutral products.

Meta- and para-chlorophenyl anions are readily generated in the gas phase from the reaction between fluoride ion and meta- and para-chlorophenyltrimethylsilane, respectively. The proton affinities of these anions were bracketed between furanide ion and hydroxide. Therefore, we assign the gas phase acidity, ΔH°acid, of the meta- and para- positions of chlorobenzene to be 390 ± 2 kcal mol⁻¹. Ortho-chlorophenyl anion is selectively prepared from the deprotonation of chlorobenzene by furanide ion. Bracketing experiments locate the ΔH°acid of the ortho-position of chlorobenzene to be 388 ± 2 kcal mol⁻¹. Each of these ions undergoes efficient CID to give chloride as the only product. The threshold energies found for chloride loss from the three chlorophenyl anions are 16, 24, and 35 kcal mol⁻¹ (± 3), respectively. The acidities and activation energies measured in this study can be combined with the literature thermochemistry for CI' and chlorobenzene to obtain absolute heats of formation for the isomeric benzynes: ΔH°f(ortho-benzyne) = 106 ± 3 kcal mol⁻¹, ΔH°f(meta-benzyne) = 116 ± 3 kcal mol⁻¹, and ΔH°f(para-benzyne) = 128 ± 3 kcal mol⁻¹. These results are summarized in table 1.

The same approach was used for the dehydrotoluenes. Deprotonation of ortho-, meta-, and para-chlorotoluenes gives ortho-, meta-, and para-chlorobenzyl anions which undergo efficient chloride loss upon CID to give α,2-, α,3-, and α,4-dehydrotoluene, respectively. The threshold energies obtained can be combined with literature values for meta- and para-chlorotoluene acidities and the bracketed acidity for ortho-chlorotoluene (ΔH°acid = 374 ± 3 kcal mol⁻¹) to give heats of formation for the dehydrotoluenes: ΔH°f(α,2-dehydrotoluene) = 106 ± 4 kcal mol⁻¹, ΔH°f(α,3-dehydrotoluene) = 96 ± 3 kcal mol⁻¹, and ΔH°f(α,4-dehydrotoluene) = 112 ± 4 kcal mol⁻¹. A notable result obtained here is that the isomer responsible for the
CID

\[
\Delta H_f(C_6H_4) = (E_T + 0.6) - \Delta H_f(Cl^-) + \Delta H_{o-c}(C_6H_6Cl) + \Delta H_f(C_6H_6Cl) - \Delta H_f(H^+)
\]

<table>
<thead>
<tr>
<th></th>
<th>ortho</th>
<th>meta</th>
<th>para</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E_T)</td>
<td>0.68 ± 0.13</td>
<td>1.05 ± 0.13</td>
<td>1.53 ± 0.13</td>
</tr>
<tr>
<td>kcal mol(^{-1})</td>
<td>15.7 ± 3.0</td>
<td>24.2 ± 3.0</td>
<td>35.3 ± 3.0</td>
</tr>
<tr>
<td>(\Delta H_{old})</td>
<td>388 ± 2</td>
<td>390 ± 2</td>
<td>390 ± 2</td>
</tr>
<tr>
<td>kcal mol(^{-1})</td>
<td>106 ± 3</td>
<td>116 ± 3</td>
<td>128 ± 3</td>
</tr>
</tbody>
</table>

Table 1

Biological activity of the neocarzinostatin chromophore, \(\alpha,3\)-dehydrotoluene, is lowest in energy of the three dehydrotoluenes.

Other interesting thermochemical data can be derived from the measured heats of formation. For example, using the currently accepted value for a C-H bond dissociation energy in benzene, 110.9 ± 2.0 kcal mol\(^{-1}\), it is possible to calculate the C-H bond strengths for a phenyl radical. With the heats of formation reported here, the following C-H bond strengths are obtained: \(\Delta H_f(ortho-C_6H_4-H) = 80\) kcal mol\(^{-1}\), \(\Delta H_f(meta-C_6H_4-H) = 90\) kcal mol\(^{-1}\), and \(\Delta H_f(para-C_6H_4-H) = 102\) kcal mol\(^{-1}\), with all the C-H bonds in phenyl radical being weaker than the C-H bond in benzene. A similar calculation can be made for the dehydrotoluene system to give the C-H bond energies for benzyl radical. In this case, the ortho- and meta- C-H bond energies are nearly the same as that in benzene, while the meta carbon-hydrogen bond is 10 kcal mol\(^{-1}\) lower than a benzene C-H bond.

Finally, we have been able to prepare the distonic para-benzyne radical anion via CID on para-trimethylsilylphenyl anion. The ability to generate this and other remote radical anions provides a route to determining electronic properties for the neutral diradicals.

References

Beams of neutral molecules can be formed in the mass spectrometer by neutralization or dissociation of mass selected ions. Reionization of these neutrals leads to neutralization-reionization (NR) mass spectra which can reveal useful information on the structures of the mass-selected parent ion and the intermediate neutral species, as well as on the fragmentation mechanism(s) of the parent ion. These capabilities of neutralization-reionization mass spectrometry (NRMS) are illustrated here with specific examples.

**Ion Structures.** The collisionally activated dissociation (CAD) spectrum of protonated acetaldehyde shows prominent C$_2$H$_3$+ and OH$_2$+ fragments that are not observed in the corresponding NR spectrum (Figure 1). This is consistent with the presence of two structures in the C$_2$H$_5$O+ parent ion beam, namely the conventional CH$_3$CH=OH+ ion and the proton bound complex C$_2$H$_2$H+—OH$_2$, formed from the former via H rearrangement. The complex can yield C$_2$H$_3$+ and OH$_2$+ upon CAD, but decays into C$_2$H$_2$, H-, and OH$_2$ upon neutralization, thus disabling the formation of C$_2$H$_3$+ and OH$_2$+ after reionization.

![Figure 1](image)

**Neutral Structures.** NR spectra provide data on the stability of the neutrals accessed upon neutralization. E.g., that of figure 1B contains a significant recovered parent ion (m/z 45) showing...
that the radical CH$_3$CH-OH survives at least partly intact and must therefore have an appreciable barrier towards dissociation. The insignificant abundances of OH$^+$ (m/z 19) and C$_2$H$_5^+$ (m/z 27) in the NR spectrum further prove that the van der Waals complex C$_2$H$_5$H--OH$_2$ decomposes before it can be reionized (lifetime <<<0.11 μs).

A different radical, CIHCl$^-$, in which a light atom is surrounded by two heavy atoms, is hypervalent but nevertheless predicted by theory to be "vibrationally bound." CIHCl$^-$ can be formed in the gas phase by neutralization of CIHCl$^-$; upon reionization into anions no recovered CIHCl$^-$ is observed, indicating that CIHCl$^-$ is unstable in the time frame of the experiment (lifetime <<<0.2 μs). Similar conclusions were drawn by Holmes et al. from CIHCl$^-$ reionization into cations.

**Fragmentation Mechanisms.** Conventional MS/MS spectra contain only the ionic fragments from a specific parent ion. The simultaneously formed neutral fragments can be detected after reionization. From the resulting NR spectra knowledge is gained on the fragmentation mechanism(s) of the parent ion.

**Figure 2.** (A) CAD, He (ionic fragments) and (B) NR, He/O$_2$, spectra (neutral fragments after O$_2$ reionization) from protonated leu-enkephalin (Tyr-Gly-Gly-Phe-Leu).

Figures 2A and 2B show the ionic and neutral fragments, respectively, generated from the protonated pentapeptide leucine-enkephalin upon CAD. Despite the limited resolution in the NR spectrum, the neutral products from important N- and C-terminal fragmentations can be recognized: 131 Da (Leu residue) from MH$^+$-$\rightarrow$ b$_4$; 147 Da (Phe residue, largest neutral loss) from b$_4$-$\rightarrow$ b$_3$; 57 Da (Gly residue) from b$_3$-$\rightarrow$ b$_2$ (also from y$_4$-$\rightarrow$ y$_3$); 119 Da (Phe residue) from a$_4$-$\rightarrow$ b$_3$; and 135 Da (Tyr residue) from MH$^+$-$\rightarrow$ y$_4$ (+ CO). The absence of high mass peaks in the NR spectrum indicates that dipeptide or larger units are not eliminated directly from MH$^+$. The smaller backbone fragments are rather formed from the bigger via consecutive losses of single amino acid residues. Internal fragment ions are also observed in the CAD spectrum (e.g., m/z 205 and 177). These products do not originate by a rearrangement in which the N- and C-termini approach each other and are eliminated as a single piece because such a neutral fragment (351 Da) is not observed in the NR spectrum. Their formation must proceed through a stepwise mechanism: MH$^+$-$\rightarrow$ y$_4$-$\rightarrow$ y$_3$-$\rightarrow$ m/z 205 (loss of 131 Da) $\rightarrow$ m/z 177 (CO loss). Finally, the intense ions around m/z 90-92 and 106-108 in the NR spectrum can arise from losses of the aromatic side chains or from the further dissociation, after reionization, of eliminated phenylalanine and tyrosyl residues.
PHOTODISSOCIATION OF n-HEPTANE MOLECULAR ION: AN INVESTIGATION OF CONSECUTIVE REACTION IN REAL TIME

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Photodissociation (PD) of n-heptane molecular ion has been studied using the mass-analyzed ion kinetic energy spectrometry (MIKES). Even though competing consecutive reactions are usually invoked to explain mass spectral data, cases for direct investigation of consecutive reactions have been rare. For example, Baer and coworkers determined the rate constant for the secondary reaction in the consecutive reaction of halobutane molecular ion on a microsecond time scale using photoelectron-photoion coincidence technique.[1] Tandem mass spectrometry has been used for indirect identification of consecutive channels. In the present study, consecutive dissociation pathways have been observed in real time and rate constant for a consecutive reaction has been determined on a nanosecond time scale using the PD technique developed recently.[2]

The collision cell assembly of the ZAB-E mass spectrometer located in the second field free region was modified to produce an electric field of \(-5 \sim 5 \text{ kV/cm}\). The laser (Argon ion) beam was made to cross the ion beam at a position within the field. Then, analyzing the translational energy of daughter ions, time-resolved investigation of a consecutive reaction was possible. A time resolution of around 1 ns could be achieved. Charge exchange ionization (CS2/N2) was used to generate the molecular ion of n-heptane with a rather well defined internal energy.

Figure 1a shows a PD/MIKE spectrum for n-heptane molecular ion. Appearance of several peaks suggests that competing reactions participate in the PD of n-heptane molecular ion. Figure 1b shows a field-on PD/MIKE spectrum for n-heptane molecular ion, which was obtained with the applied voltage on the cell. Symmetric nature of the peaks with m/z 56, 57, 70, and 71 implies that the total dissociation rate constant of the photoexcited n-heptane molecular ion is larger than the maximum value \(k_m \sim 10^9 \text{s}^{-1}\) resolvable with the present instrument. On the other hand, the PD/MIKE signal for m/z 43 consists of two peaks designated as A and B in Fig. 1b. Moreover, the peak A is asymmetrically broadened and tails toward the peak B. Detailed analysis showed that this unexpected spectral feature resulted from the consecutive reaction 100+ → 71+ → 43+ under two assumptions: (1) The total rate constant is larger than \(k_m\). (2) The rate constant for the secondary reaction is smaller than \(k_m\).

In addition, several other consecutive channels could be identified, even though those were not as efficient as above: 100+ → 85+ → 43+, 100+ → 57+, and 100+ → 55+. Considerably broad rate constant distribution for the secondary reaction was obtained. This may be originated from the broad internal energy distribution of the intermediate ion due to the energy partitioning in the primary dissociation. Average rate constants for the consecutive

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reaction determined were $(3.3 \pm 1.0) \times 10^7$ and $(1.88 \pm 1.3) \times 10^7 \text{s}^{-1}$ for PD at 488.0 and 514.5 nm, respectively. The PD channels of \( n \)-heptane molecular ion are shown as in Scheme 1. Based on rough estimations, the fragmentation pathways determined in this work including the consecutive reactions have been found to be in agreement with the statistical expectation.

![Scheme 1. Photofragmentation pathways for \( n \)-heptane molecular ion.](image)

References

The 39th ASMS Conference on Mass Spectrometry and Allied Topics

MASS SPECTROMETRY OF BUCKMINSTERFULLERENE AND ITS CONGENERS

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Abstract

It is well-known that a graphite vaporized by laser irradiation \(^1\) in a helium atmosphere yields ionized C\(_{60}\) and C\(_{70}\) in high abundance. It is also well-known that a high-power AC arc applied between graphite rods \(^2\) in a helium atmosphere produces macroscopic amount of buckminsterfullerene, C\(_{60}\), and its C\(_{70}\) analog in a few % yield. We now report our study on graphitic soot (product of the arcing process) using mass spectrometry. Extracts of graphitic soot are shown to contain, in addition to the well-known fullerenes C\(_{60}\) and C\(_{70}\), smaller quantities of much larger carbon clusters, up to C\(_{124}\). These compounds were detected by electron attachment and electron impact mass spectrometry (Figure 1). The relative stabilities of C\(_{60}\), C\(_{70}\), and C\(_{54}\) were investigated by charge exchange and collision activated dissociation experiments. The positively and negatively charged ions showed remarkable stability to fragmentation, although the reactions C\(_{70}\) \(^+\)\(\rightarrow\)C\(_{54}\)\(^+\) and C\(_{70}\) \(^-\)\(\rightarrow\)C\(_{54}\)\(^-\) provide evidence for interconversion of fullerenes. The data show the conspicuous absence of particular clusters.

Mass spectra of positive and negative ions derived from the fullerenes C\(_{60}\) and C\(_{70}\) show persistent satellite peaks. Differential evaporation into the mass spectrometer and changes in relative ion abundances with sample preparation suggest that the satellite ions are not generated in the mass spectrometer but correspond to four stable species with 14, 15, 16, and 17 additional mass units that are produced during the synthesis of the fullerenes or their extraction from graphitic soot. They are assigned as adducts of C\(_{60}\) and C\(_{70}\) containing CH\(_2\), CH\(_3\), O\(_2\), and OH, respectively. Collision activated dissociation of the (M+17)\(^+\) and (M+16)\(^+\) (M = C\(_{60}\), C\(_{70}\)) adducts yielded the respective ions of C\(_{60}\) and C\(_{70}\). Irradiation, using a mercury arc lamp, of mixtures of C\(_{60}\) and C\(_{70}\) increased the yields of the C\(_{60}\)+O and C\(_{70}\)+O adducts. In addition, products that corresponded to (C\(_{60}\)O + (CH\(_2\))\(_n\)) where n = 1,2,3,4,5, and (C\(_{70}\)O + (CH\(_2\))\(_n\)) where n = 1,2 occurred on irradiation of solutions of soot extracts (Figure 2). These compounds were absent upon irradiation of fullerene mixtures that were thoroughly washed with ether. Irradiation of the ether wash containing small amounts of C\(_{60}\) and C\(_{70}\) and the monoxides gave increased yields of C\(_{60}\)O and C\(_{70}\)O as well as C\(_{60}\)O\(_n\), where n = 2,3,4,5 and C\(_{70}\)O\(_n\), where n = 2 (Figure 3). These series of adducts are interpreted as products resulting from sequential cyclopropanations and epoxidations.

A variation of the arcing experiment was made using graphite electrodes with metallic inserts. Many low mass components were observed in the extract of the graphitic soot but none of the buckminsterfullerenes.

References

3. The ion m/z 736, generated by fast atom bombardment was shown to have the formula C\(_{60}\)O (Gross, M.L.; Caldwell, K. personal communication, March 1991).
Figure 1.

(a) Electron attachment mass spectrum of a soot extract after ether wash, showing negative ions formed using ammonia reagent gas.  
(b) Electron impact mass spectrum of the same sample.

Figure 2.

Electron attachment mass spectrum of raw soot extract after 3 hours of UV irradiation showing the \((C_{90}O+(CH_3)_n)^-\) and \((C_{70}O+(CH_3)_n)^-\) adducts.

Figure 3.

Electron attachment mass spectrum of ether washings of raw soot extract after 45 minutes of UV irradiation showing the \((C_{90}O)^-\) and \((C_{70}O)^-\) adducts.
SURFACE-INDUCED DISSOCIATION OF DISTONIC RADICAL IONS

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Distonic radical ions and their conventional counterparts have been extensively investigated both experimentally and theoretically. It was recently reported that low-energy gas-phase collisions can be used to differentiate between distonic ions and their conventional counterparts. We now report surface-induced dissociation results for distonic and conventional ions. The distonic ions investigated include \( \text{CH}_2(\text{CH}_2)_n\text{OH}^+ \) with \( n=0-2 \) and \( \text{CH}_2(\text{CH}_2)_n\text{NH}_3^+ \) with \( n=1-2 \). These ions were allowed to collide with the surface and their ion/surface collision behavior was compared with that of their conventional isomers \( \text{CH}_3(\text{CH}_2)_n\text{OH}^+ \) and \( \text{CH}_3(\text{CH}_2)_n\text{NH}_2^+ \). Deuterium-labelled distonic and conventional ions were also investigated, as was the influence of the nature of the surface (e.g. stainless steel, gold, ethanethiol and octadecanethiol on gold). The goals of the research are to determine the (i) surface-induced dissociation behavior of the distonic vs. conventional ions and (ii) the relative reactivity of the distonic vs. conventional ions toward H addition from surface-bound hydrocarbons.

With certain projectile ions, charge exchange can occur between the mass-selected projectile ion and surface adsorbates with the release of ionized adsorbates from the surface. Our data show that the use of a modified surface, consisting of a self-assembled monolayer film of ethanethiol on gold, drastically reduces the relative amount of charge transfer that occurs when polyatomic ions collide with the surface in the gas phase. This can be exemplified by ethanol and its distonic ion and in Figure 1 which compares an untreated gold surface (Fig.1a) with the ethanethiol modified gold surface (Fig.1b). These surfaces were used for the ion structure determination described below.

It was found that surface-induced dissociation spectra can be used to distinguish the distonic ions and their conventional counterparts. This can be seen in Figure 2 for ethanol and its distonic ion and in Figure 3 for ethylamine and its corresponding distonic ion. The distonic ions and their conventional isomers produce distinct spectra that indicate little isomerization. Some H addition does occur and can be seen in the fragmentation pattern in the spectra. Further studies will be required to determine whether these ions are the result of an initial neutralization followed by a proton transfer or if the distonic ions are reacting as radicals to abstract a hydrogen from surface-bound hydrocarbons.

We are developing methods for detecting volatile organics in water and soil samples. Our twofold goal is to develop rapid, fieldable screening procedures to identify contaminated samples that will require full analysis by EPA-approved methods, and to scope the utility of direct sampling MS for quantitating volatiles in the low ppb range.

The apparatus consists of a Finnigan ion trap mass spectrometer (ITMS) connected via an open-split interface with a purge device. The sample, contained in a standard 40 mL VOA vial, attaches to the purge device. The sample is sparged with helium at a flow rate of 100-150 mL/min. A portion of the purge gas flows into the ITMS through a restricting capillary. Mass spectra are obtained under El conditions.

Water standards were prepared by injecting a methanol stock solution into 25 mL of water that contains NaCl and Na2SO4 to simulate groundwater samples of interest to the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA). Soil standards were prepared by spiking 5 g of soil with a stock solution. 25 mL of water were pipetted onto soil standards before purging.

To demonstrate the screening ability of direct-sampling ITMS, water standards containing the 34 volatiles on the EPA Target Compound List (TCL) at concentrations ranging from 1 ng/mL to 250 ng/mL were analyzed. Using data obtained from analyses of the individual components, most peaks in the mass spectrum of the mixture, shown in Figure 1, could be assigned to one or more TCL compounds. The goal of our screening procedure is "no false negatives"; i.e., all TCL volatiles present at or above quantitation limits must be detected. The fact that many of the ions occurring in the mixture spectrum are due to more than a single compound is not fatal for a procedure designed to eliminate uncontaminated samples from the queue for full-blown analysis. Eventually, the construction of a library with information about multiple ions observed for many compounds will lessen this interference effect. One problem with the screening procedure is that water-soluble ketones do not purge rapidly. Less soluble volatiles yield intensity vs. purge time profiles with an initial rapid rise followed by an exponential decay, reaching baseline intensity in 3 to 5 minutes. Purge profiles for the ketones rise to a low level and do not readily return to baseline, as Figure 2 shows. This behavior leads to higher detection limits for these compounds.

Mixtures of benzene, trichloroethene and tetrachloroethene ("BTT") spiked at 50 ppb(v) into a number of water and soil matrices were analyzed to evaluate direct-sampling ITMS for quantitation. Integrated purge profile areas for characteristic BTT ions in the samples were ratioed to the corresponding areas for BTT in water to give recoveries. Adjusting the pH of water samples over the pH=2 to pH=10 range had no significant effect on BTT recovery. A high organic content in the water, simulated with a potting soil leachate prepared by filtering a mixture of potting soil and water, did not interfere with BTT recovery. In general, soil samples reduced BTT recoveries, although some of this loss is associated with the spiking procedure rather than with the analysis. Soil types examined include a commercial potting soil, (mixture of sand and organic matter), Rocky Mountain Arsenal soil (sandy), a soil provided by USATHAMA (finely-divided clay), a local Oak Ridge soil, and a Knox county soil (mixed clay and organic matter). Precision of results for the various soils depended strongly on the uniformity of the samples. The three compounds also tended to purge differently relative to one another depending on soil composition. BTT recovery from the USATHAMA soil was lower than from other soils, indicating that this soil is the most adsorptive of those tested. We also evaluated different purge conditions for the USATHAMA soil. Heating the sample during the purge increased recovery slightly, while sonicating the sample had little effect. Allowing the sample to stand for 24 hours after adding water resulted in no significant loss of volatiles. In addition to the matrix comparison at 50 ppb(v), calibration curves constructed for BTT in water over the 1 to 1000 ppb(v) range were reasonably linear in the 1 to 100 ppb(v) range, but showed some deviation from linearity at higher concentrations because of space charging.

Direct sampling ITMS is useful for screening environmental samples for volatiles. Our results show that quantitation is feasible, despite some matrix effects. We plan to study other matrices and investigate alternative methods for troublesome compounds such as water-soluble ketones.
DS-iTMS SCREENING

34 EPA Volatiles Purged from Water

Corrected Intensity (arbitrary)

\[
\begin{array}{c|c|c}
\text{Concentration (ng/mL)} & 1 \text{ ng/mL} & 10 \text{ ng/mL} & 100 \text{ ng/mL} \\
\hline
m/z & 40 & 60 & 80 & 100 & 120 & 140 & 160 & 180 & 200 \\
\end{array}
\]

Figure 1

PURGE PROFILES

Direct-Sampling ITMS

Corrected Intensity (arbitrary)

\[
\begin{array}{c|c|c}
\text{Compound} & \text{Concentration (ng/mL)} & m/z \\
\hline
\text{CHLOROBENZENE} & 100 \text{ ng/mL} & 112 \\
\text{2-METHANONE} & 1 \mu g/mL & 100 \\
\end{array}
\]

Scan Number

Figure 2


1200
A new technique has been developed to extract volatile organic compounds from aqueous systems into the gas phase. Unlike the conventional purge-and-trap method with the bubble purger, the new technique disperses the liquid sample in a flowing carrier gas. The water sample is sprayed through a nozzle into an extraction chamber (Fig. 1), where the formation of tiny droplets increases the total surface area dramatically and accelerates the partitioning process of the dissolved compounds between the aqueous sample and the gas phase. The water pressure and flow required by the spray process can be provided either by a pump or by a pressurized sampling chamber. Fig. 2 shows a pump-driven spray extraction system schematically. In the system with the sampling chamber, compressed nitrogen is used to pressurize the chamber and as carrier gas for the extraction. The sampling chamber can be dipped into water and a "normally-open" spring-loaded valve at the bottom lets the liquid enter the chamber. Whenever pressure is applied, the spring-loaded valve closes and the system becomes ready to spray. An automated valve-operation sequence controls the entire spray extraction process.

The carrier gas enters the extractor at the top (Fig. 1) and travels down through the spray cone against the flight direction of the water droplets (counter-current). At the lower part of the chamber, the gas exits through the slot of the protected exit tube. After flowing down the chamber walls, the sprayed water sample exits the extractor through the drain tube at the sink shaped bottom. In order to prevent the carrier gas from leaving the chamber through this "wrong" exit, there is always a little residual water in the sink. The volume of this "rest" is monitored by a level sensor in the chamber. As soon as the water level exceeds a pre-defined mark, the signal of the level sensor is used to open the magnetic valve in the drain tube. The drain valve stays closed when the water level is below a marked height. The carrier gas enters an automatic gas preconcentrator, equipped with a Tenax sorption tube. Subsequently, the Tenax tube is heated and the desorbed gases are transferred into the GCMS. The Mobile Environmental GC-Mass Spectrometer "HEM" of Bruker-Franzen Analytik was used.

A mixture of volatile organic compounds (VOC) has been
used to test the response of the complete system. The test results as a diagram of the VOC concentration in water versus the GC peak area is depicted in Fig. 3. The linearity of the data points is remarkable. As a natural result of the extraction process, water vapor is also entering the sorption tube. In the experiments, the Tenax tube was "dry-purged" for 30s between the sampling and the desorption events, in order to prevent large amounts of water vapor from entering the GCMS. Experiments using the spray extractor / preconcentrator / MRM (2 min. of sampling, simple flow, 900ml of water) have shown, that compounds with relative low solubility in water (benzene, toluene etc.) can be detected in a concentration range as low as 10-30 ng/l. The spray extractor can also be operated as a stand-alone device. Extracted compounds can be captured in a Tenax tube mounted on the gas exit. They can then be analyzed separately.

The spray-and-trap method as a continuous monitoring technique can be applied to large-size water samples like industrial waste waters, river, lake and seawater in the simple flow mode: The water sample passes through the extractor only once. However, for small-volume samples, the circulation mode is recommended. A calibration of the sampler for each different mode is necessary.

Increased curvature of the surface (droplets!) leads to an increase of vapor pressure of the liquids (Kelvin equation): $P_{\text{mist}} = P_{\text{bulk}} \exp\left(\frac{2\gamma V_m l}{\pi r^2 T}\right)$. $V_m$ is the molar volume, $r$ the droplet radius and $\gamma$ the surface tension. This effect, supporting the extraction, is however small and becomes only remarkable at sub-micron sized water droplets.

The spray-and-trap method can be applied to solutions containing surfactants. Unlike in the bubble purger, the operation of the system is not blocked by a foam formation. Some effects of surfactants are observed in the quantitative analysis data from spray extraction (usually minor and compound dependent). Standard addition method is recommended to increase accuracy.

1) Patent applied for
The determination of specific organic chemicals in natural waters is of prime importance in the assessment of the environmental impact of waste liquid effluent discharges. Conventionally, these measurements are performed by discrete sampling of the waterbody followed by specific analysis at the Laboratory by methods such as gas chromatography and gas chromatography-mass spectrometry. While this process provides a considerable amount of useful data, it is comparatively slow and there is a real need for a method which is capable of direct determination of these substances in the field.

In conjunction with VG Gas Analysis Systems Ltd, this Laboratory has investigated the potential of membrane inlet mass spectrometry as a field method. This technique has previously been used by a number of workers to measure volatile organic chemicals in aqueous samples and is currently undergoing a resurgence in the United States. Beginning in 1985, we have carried out a number of field surveys of natural waters with a small portable mass spectrometer fitted with a membrane inlet, the VG “AquaPetra”. Originally, the system was transported to the banks of the river estuary by mobile van where the constituent parts were assembled. These included a pumping system to bring the estuarine water into the vicinity of the mass spectrometer so that it could be sampled by its own flow system. Using this apparatus, information on the concentrations of a number of organic chemicals in the estuarine water was obtained by monitoring up to 16 selected ions.

Although, this mode of operation did provide useful information, the system could only be used for continuous monitoring over a very restricted area. Consequently, more recent surveys have sought to extend the range of applicability by mounting the apparatus in a boat. Two surveys of this type have been carried out, the first on a relatively narrow waterway and the second on a wide estuary.

Typical results from these two surveys are shown in Figures 1 & 2, although these represent only a very small fraction of the data that was collected in each case. Figure 1 shows the results of continuously monitoring the m/z 78 ion, to represent benzene concentration, over a 16 kilometre stretch of the waterway at 2m (nominal) depth. At the beginning of the profile, the intensity of the ion is found to decrease over the first few kilometres. But at 8 kilometres, which represents the most industrialised part of the waterway, two distinct peaks are observed corresponding to discharges from petrochemical establishments. On completion of this profile, some depth profiling (0 - 7.5m) was carried out and this shows the water to be relatively well mixed at this point. The second example, Figure 2 shows a profiling run conducted in a large estuary for toluene using the m/z 92 ion to represent toluene concentration. The system was calibrated using a 100 ug/l toluene standard at both start and finish of the profiling work. After the first of these standards, a longitudinal profiling run (nominal depth 1m) was conducted over a 4 kilometre stretch of the estuary and this showed little evidence of the presence of toluene. On returning (at depths of between sub-surface and 1m), a submerged pipeline was passed and the toluene discharge from this was clearly discerned. This area was then criss-crossed to take other measurements of the toluene content of the water immediately surrounding this discharge. However, this process was complicated by the difficulty in ascertaining the relevant location since the discharge boil was only visible at intervals. Nevertheless, it was observed on a number of subsequent occasions and was monitored as is shown in the figure. The maximum toluene concentration observed in this work was approximately 20 ug/l.

Although only a small fraction of the data has been presented, the capability of membrane inlet mass spectrometry for the continuous monitoring of certain chemicals in water is clearly demonstrated. However, the high cost of the instrumentation, approximately £40,000 and its rather limited compound range, i.e to those substances of high aqueous volatility, have probably restricted its use to date.
References.


Figure 1. Profile of benzene (m/z 78) in waterway from boat based survey.

Figure 2. Profile of toluene (m/z 92) in estuarine water from boat based survey.
DIRECT MEASUREMENT OF VOLATILE ORGANICS IN AIR USING AN ION TRAP MASS SPECTROMETER

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Introduction
Growing concern over indoor air pollution, occupational exposure to airborne toxics, excessive stack emissions, accidental spills, and other sources of emission which contribute to air contamination, has led to an increased need for fast, sensitive, and selective methods for the detection of trace organics in air. In response to this need, we have been working on the application of ion trap mass spectrometers to real-time, continuous monitoring of volatile organic compounds in air.

Experimental
All experiments were performed with a Finnigan MAT ITMS ion trap mass spectrometer equipped with an electropolished vacuum chamber and dual 330 L/sec turbomolecular pumps. A special sniffer probe was designed for direct air monitoring which includes an open/split capillary restrictor interface with a 2 L/min air sampling pump attached to the split vent. This pump provides active sampling at a high flow rate, while the open/split interface maintains the pressure inside the mass spectrometer at a tolerable level. Normally, the low mass cutoff for the ion trap is set at or above mass 35 in order to eliminate water and air ions from the cell. Under these conditions, either electron impact or chemical ionization (using a reagent such as isobutane) can be used to ionize the analytes. Typically, conventional full scan electron impact mass spectra are acquired and searched for the presence of characteristic ions which may correspond to the presence of target analytes. For greater confidence in the identification of an analyte, the ITMS may be operated in a selected ion storage MS/MS mode.

In order to establish the detection limits of the instrument, trace levels of volatile organics in air were generated using a syringe pump vapor generator. This device continuously meters a known volume of a dilute solution of volatile organics into a flowing air stream. The concentration of the analytes can be controlled by altering either the flow rate of the air or the amount of analyte metered into the air stream. Using this apparatus, reliable concentrations VOC's in air can be generated ranging from low ppb levels to greater than 10 ppm. Certified standards of volatile organics in nitrogen were also purchased and used to confirm the proper operation and calibration of the vapor generator.

Results and Discussion
An important aspect of successful direct air sampling with our ITMS is that the helium buffer gas (for the stabilization of ions in the cell) is added to the instrument through the air sampling probe instead of by means of a batch inlet. This improves the overall performance of the instrument in terms of resolution, reduced multiplier noise, and better detection limits as shown in Figure 1. In order to minimize dilution of the air sample with the helium, a pulsed valve is used to inject helium into the sampled air stream at a rate of approximately twice per second. Using a sniffer probe approximately 6 feet long in conjunction with the 2 L/sec air sampling pump, the response time of the ITMS is generally less than 5 seconds.

Under electron impact ionization conditions, the real-time detection limits for 30 different volatile organics in air have been found to range from approximately 2 ppb to 50 ppb (Table 1). Linear calibration curves have been generated over a 2-3 order of magnitude concentration range (without the use of the Automatic Gain Control software) indicating that quantification of target analytes is feasible at low ppb concentrations (Figure 2). Although extensive experiments have not yet been performed for a wide range of VOC's, preliminary experiments indicate that detection limits in the part-per-trillion range may be achieved for some compounds through the use of chemical ionization reagents.
Laboratory tests of the ITMS indicate that it can be configured for the real-time monitoring of volatile organic compounds at low ppb concentrations. Quantification is possible either by the addition of a standard to the sample or by comparison of instrument response with an external calibration curve. Electron impact ionization provides universal detection, while chemical ionization can be used for more selective ionization and in some cases improved detection limits. Further, the MS/MS capability of the ITMS provides an important added dimension to the identification of target analytes in complex samples. At this time, a fieldable version of the air monitoring ion trap is under construction and is scheduled for initial testing later this year.

### VOC Detection Limits in Air

<table>
<thead>
<tr>
<th>Compound</th>
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<tr>
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<tr>
<td>1,1,1-Trichloroethane</td>
<td>2</td>
</tr>
<tr>
<td>Toluene</td>
<td>3</td>
</tr>
<tr>
<td>Methylene Chloride</td>
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</tr>
<tr>
<td>Acetone</td>
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<td>2-Butanone</td>
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</tr>
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<td>1,1-Dichloroethane</td>
<td>18</td>
</tr>
<tr>
<td>O-Xylene</td>
<td>4</td>
</tr>
<tr>
<td>Carbon Disulfide</td>
<td>25</td>
</tr>
</tbody>
</table>

DETECTION OF EXPLOSIVES RESIDUE
BY ION MOBILITY SPECTROMETRY

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The United States has been a prime target of international terrorism for at least two decades. The bombings of the U.S. Embassy and the U.S. Marine Barracks in Beirut in 1983, and the destruction of Pan American Flight 103 over Lockerbie, Scotland in 1989 are grim reminders of terrorism in recent years. These criminal acts have prompted the need for the development of new and innovative methods of explosives detection. As part of an ongoing research effort, the FBI Laboratory has been evaluating new technology and the innovative adaptation of existing technology for use in counterterrorism and counternarcotics investigations.

The widely varying (ppm to ppt) vapor pressures of explosives hidden in packages, luggage or on people requires extremely sensitive and selective detection methods. Laboratory studies have shown that explosive material can be transferred from contaminated hands to surfaces. Therefore, the sampling of particulate residue on surfaces offers a greater chance for detection of concealed explosives.

Three commercially available ion mobility spectrometers (IMS); an IONSCAN (Barringer Instruments, Inc., West Plainfield, NJ); a Phemto-Chem PC-110 (FCP, Inc., West Palm Beach, FL) and a briefcase sized Graseby PD-5 (Graseby Dynamics Ltd., Watford, Herts, England) are being evaluated for routine forensic applications.

The IONSCAN collects explosives residue on a membrane filter while the PC-110 uses a quartz wool preconcentrator tube. In both cases sample collection, thermal desorption, and analysis requires less than 30 seconds. The Graseby PD-5 is designed for direct air sampling and is therefore only sensitive to the more volatile nitrated ester explosives, ethyleneglycol dinitrate (EGDN) and nitroglycerine (NG).

Sampling procedures for examining packages, luggage and people for hidden explosives have been developed for each of the detectors. Laboratory evaluation of the IONSCAN system has determined the limits of detection for common explosives to be 50-600 pg. Figure 1 show the identification of a PETN based sheet explosive. The PC-100 IMS has a limit of detection of 0.03 ppt RDX vapor. From a saturated head space vapor, a 10 second air sample results in the detection of 20 fg RDX as shown in Figure 2. For the Graseby PD-5, 0.25 pounds of dynamite can readily be detected in a cardboard box from 10-12 feet after a "soak time" of 1 hr.

The IMS detectors have been employed in actual terrorist investigations. Following a domestic dispute, a local police agency asked the FBI Laboratory to identify homemade nitroglycerine in a basement prior to it being rendered safe. Using the Graseby PD-5 the NG was safely located and identified. In another incident, the PD-5 was used to assist the FBI and New York City Police Joint Terrorist Task Force in a narcoterrorist investigation by searching 1,300 rental storage lockers and 6 apartment buildings for dynamite.

The recent Middle East crisis heightened the world's awareness to the potential for terrorist attacks. In January, 1991 all three IMS detectors were employed by the FBI Laboratory to provided added security and to respond to potential terrorist threats during Superbowl XXV in Tampa, FL. Figure 3 shows the detection of RDX on the trunk of a car which had C-4 explosive hidden in it for testing and training of canines.

This is a publication of the Laboratory Division of the Federal Bureau of Investigation. The use of names of commercial manufacturers does not constitute or imply endorsement, recommendation or favoring.
Figure 1. Identification of a PETN based sheet explosive (peaks 5-7) using an IONSCAN IMS System. The drift time and reduced mobility of the ions are displayed to the left of the plasmagram.

Figure 2. Detection of 20 fg of RDX vapor using a PC-110 IMS system. Saturated headspace vapor was collected on a quartz wool preconcentrator.

Figure 3. Detection of RDX (peaks 2-4) explosive on the trunk of a car used to store C-4 for canine testing and training. The IONSCAN system was used.
DIRECT INTRODUCTION OF PESTICIDE-CONTAMINATED CROP SAMPLES TO A MASS SPECTROMETER VIA A PYROLYSIS PROBE

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A. Peter Snyder, U.S. Army Chemical Research, Development and Engineering Center, Aberdeen, MD

Pyrolysis is an established method for introducing complex samples such as natural and synthetic polymers, commercial drug and pesticide formulations and intact microorganisms to a gas chromatograph or mass spectrometer. We are investigating the use of pyrolysis in conjunction with mass spectrometry for the detection of pesticide residues in crop matrices. The potential advantages of this approach are reduction of the amount of extraction and cleanup needed and the ability to detect unextractable bound residues.

We have chosen as representative pesticides four triazine compounds (fig. 1) used extensively in Florida agriculture. Simazine, atrazine and ametryn are herbicides used for the protection of citrus and other crops; cyromazine is an insecticide used for the protection of several minor crops including Chinese cabbage. Bok Choy Chinese cabbage was used as a representative matrix for cyromazine and orange peel was used for the three herbicides.

Data were obtained using a Sciex TAGA 6000 atmospheric pressure ionization (API) triple-quadrupole mass spectrometer. Samples were introduced to the mass spectrometer via a Pyroprobe Model 122 Controller equipped with a platinum coil desorption probe (Chemical Data Systems, Oxford, PA) ramped to 600°C at 240°C/min. 5-mg crop samples were spiked in situ with pesticide analytes at concentrations ranging from 400 ppm to 1 ppm.

Under these conditions, all four analytes evaporated from the probe without decomposition giving API mass spectra dominated by MH⁺ (fig. 2). Crop samples, on the other hand, underwent thermal degradation to volatile products of low molecular weight (fig. 3). Even in the absence of chromatographic separation, some temporal resolution of analytes and substrates was seen in spiked samples (fig. 4). Further enhancement of signal-to-noise was sought by obtaining CID daughter-ion spectra of MH⁺ for the four analytes. In each daughter ion spectrum three diagnostic ions were identified as XCNC(NH₂)NH⁺, CHNX⁺ and CH,N₂X⁺, where X=Cl for simazine and atrazine, X=SCH₃ for ametryn and X=NH₂ for cyromazine. Selected reaction monitoring for these three daughter ions permitted detection of low-ppm levels of each analyte in spiked crop samples (Table 1). Efforts are underway to lower these LOD’s by the introduction of chromatographic separation.

REFERENCES

Figure 1. Structures of four representative triazine pesticides: simazine (mol. wt 201), atrazine (mol. wt 215), ametryn (mol. wt 227) and cyromazine (mol. wt 166).

Figure 2. Thermolysis-API mass spectra of (a) simazine, (b) atrazine, (c) ametryn and (d) cyromazine.

Figure 3. Py-API mass spectra of (a) bok choy Chinese cabbage and (b) orange peel.

Figure 4. Time-intensity profile for m/z 167 in the Py-API mass spectrum of (a) 8 mg bok choy Chinese cabbage and (b) 5 mg bok choy spiked with 2 µg cyromazine.

| TABLE I |
| Limits of detection for triazine pesticides on 5 mg agricultural matrices by Py-API-MS/MS using multiple reaction monitoring: |
| Atrazine/orange peel: 60 ppm |
| Ametryn/orange peel: 16 ppm |
| Cyromazine/bok choy leaf: 4 ppm |
| Simazine/orange peel: 1 ppm |

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SELECTIVE DETECTION OF CHLORINE-CONTAINING COMPOUNDS IN A COMPLEX MIXTURE

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Introduction

The addition of a reaction gas to a low pressure microwave-induced plasma (MIP) creates a reaction interface in which complex molecules are converted into small stable neutrals. For a given reaction gas used the molecules formed represent the elemental composition of the original analyte. In this study SO₂ has been used as a reaction gas for the selective detection of chlorine-containing compounds in a complex mixture, using capillary gas chromatography-microwave induced plasma-reaction interface mass spectrometry (GC-MIP-RIMS).

Experimental

The GC-MIP-RIMS apparatus is described before therefore only a brief description is given here. The GC-RIMS set-up consists of a Varian 3400 GC with a 30m DB-5 capillary column connected to a 4.5" long, 1/16" i.d. and 1/4" o.d. ceramic tube using conventional vacuum components. The ceramic tube extends through the microwave cavity. The other end of this ceramic tube is connected to a deactivated uncoated wide bore fused silica capillary which is used as a transfer line. The union on the column side of the ceramic tube was modified by adding a 1/16th inch stainless steel tubing to allow flow of the reaction gas into the ceramic tube using a leak valve. The detector was a Finnigan 4000 quadrupole mass spectrometer with INCOS, version 4.07.82, data system. The microwave chamber was powered by a 100 W, 2450 MHz power supply with a forward power of 80 W and a reverse power of 6 W. Helium was used as a carrier gas for the GC and the MIP.

Results and Discussion

Selectivity: When SO₂ was used as a reaction gas, carbon predominantly formed CO m/z 28 and CO₂ m/z 44. The chlorine primarily formed HCl m/z 36, plus some other minor components, e.g., SCI m/z 67 and SCI₂ m/z 102. At m/z 36 detection of chlorinated compounds was completely selective. This was demonstrated using dichlorobenzene and n-dodecane as the chlorinated and non-chlorinated compounds as shown in Figure 1.

Sensitivity: The GC-MIP-RIMS limits of detection for dichlorobenzene is 10 picograms on column with a S/N>3. This was achieved using single ion detection mode with 1000 ms scan time. The linear dynamic range of the calibration curve using dodecane as the internal standard is at least 4 orders of magnitude, as shown in Figure 2.
Applications in Environmental Studies A mixture of the PCB mix and several non-chlorinated compounds was used to show the selectivity for the chlorine-containing compounds in a complex mixture. The result is shown in Figure 3. As is shown m/z 36, HCl, is completely selective. After identifying the chlorinated peaks in the chromatogram of the complex mixture using the MIP-RIMS method, the experiment was repeated this time MIP was turned off and mass spectrum in the full scan mode were obtained. Figure 4 shows the mass spectra of one the identified PCBs.

Conclusion

The GC-MIP-RIMS method of chlorine detection can be used as a time saving analytical tool in the environmental testing industry. The setup allows the versatility of prescreening complex mixtures for chlorinated compounds. This is a valuable asset to the detection of PCBs, pesticides and other life threatening chlorinated compounds. Low limit of detection and wide dynamic range of the GC-MIP-RIMS, its minimal cost and its ease of operation make this setup an attractive analytical tool for the selective detection of chlorine-containing compounds and their identification in a complex mixture.

Analysis of Isomeric Alcohol Mixtures Using GC-Matrix Isolation-FTIR-MS

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University of California, Riverside
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INTRODUCTION

Isomeric alcohol mixtures are very important because of their use in various commercial manufacturing processes. The extent of carbon branching in isomeric alcohol mixtures determines their overall physical and chemical properties. Short chain alcohol mixtures are often used in plasticizer production. New legislation is requiring manufacturers to identify more specifically the constituents in their products, as well as in their products' precursors. For these reasons, the ability to separate and identify the individual constituents of commercial isomeric alcohol samples has recently become much more important.

Gas chromatography/matrix isolation infrared spectroscopy-mass spectroscopy (GC/MI-FTIR-MS) is a highly sensitive and specific technique that combines the capabilities of capillary column gas chromatography for separating components of complex mixtures with the high sensitivity and specificity of both matrix isolation infrared and mass spectrometric detection. This technique provides three distinct types of chemical information. First, the flame ionization detector provides retention times and quantitative information for each separated component of the complex mixture. Second, the matrix isolation Fourier transform infrared spectrometer provides functional group and isomer specific information. Third, molecular weight and fragmentation patterns are obtained from the mass selective detector. GC/MI-FTIR-MS is well suited for the analysis of isomeric alcohol mixtures. In this paper, application of this methodology to the analysis of two representative alcohol mixtures is described.

EXPERIMENTAL

The GC/MI-FTIR-MS instrument utilizes a Hewlett-Packard 5890 gas chromatograph with a 50:35:15 three way splitter to the IR, MS and FID, respectively. The matrix isolation Fourier transform infrared spectrometer used is a Mattson Instruments Cryolect 4800, while the mass spectrometer is a Hewlett-Packard 5970B mass selective detector. A 60m DB-wax column is used for the separations. Quantitative $^{13}$C NMR is also utilized.

RESULTS

The two samples analyzed are thought to contain only saturated, C-7 primary alcohol isomers. There are a total of 17 $\text{C}_7\text{H}_{16}\text{O}$ alcohol isomers. FID and $^{13}$C NMR data indicate the presence of more than 12 distinct isomers in each sample. Each sample contains only 7 components in sufficient concentration (>0.5%) to be simultaneously identified by IR, MS and FID. Six of the seven major components occur in both samples, although in varying relative concentrations. The individual mass and matrix-isolated infrared spectra have been recorded for all eight of the major components. Figure 1 shows the mass spectrum for 2,4-dimethylpentanol, component 2. A detailed analysis of both the mass and infrared spectra allows structure assignments to be made for each component. Table 1 shows the structural assignments for each component in order of elution (column 1), based upon the MS and MI-IR data alone, (columns 2 and 3), and the assignment based upon complimentary use of both the IR and MS data (column 4).
Figure 1. Mass Spectrum of 2,4-dimethylpentanol, Component #2.

Table 1. Identity of Isomeric Alcohol Mixture Components.

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<tr>
<th>Order</th>
<th>MS Identification</th>
<th>IR Identification</th>
<th>Proposed Structure</th>
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<tr>
<td>1</td>
<td>2,2-dimethylpentanol</td>
<td>2,2-dimethylpentanol</td>
<td>2,2-dimethylpentanol</td>
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<tr>
<td></td>
<td></td>
<td>or 2,3-dimethylpentanol</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td>or 2,4-dimethylpentanol</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2,4-dimethylpentanol</td>
<td>2,4-dimethylpentanol</td>
<td>2,4-dimethylpentanol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or 3,4-dimethylpentanol</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2,3-dimethylpentanol</td>
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</table>

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THE ATMOSPHERIC STABILITY OF POLYBROMINATED DIBENZOP-DIOXINS AND DIBENZOFURANS

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University of North Carolina at Chapel Hill
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Introduction. Atmospheric emissions of polybrominated dibenzo-p-dioxins (PBDDs) and dibenzofurans (PBDFs) are likely to increase in the future due to the rising use of brominated organic compounds in consumer products and of incineration as a means of municipal waste disposal. Information on the atmospheric stability of PBDDs and PBDFs is thus needed for risk assessment since human exposure takes place through atmospheric transport and depositional processes. Previous laboratory studies of the photodegradation of PBDDs and PBDFs have shown rapid degradation in organic solution (for 2,3,7,8-tetrabrominated dibenzo-p-dioxin $t_{1/2} = 0.8$ min) but much slower degradation on quartz surfaces ($t_{1/2} = 32$ hours for 2,3,7,8-TBDD) (1). It is unknown whether these half-lives represent actual degradation rates in the ambient environment.

Experimental. In this study dilute emissions from the combustion (640-760°C) of polyurethane foam containing polybrominated diphenyl ethers (PBDPEs) were introduced into 25 m³ outdoor teflon film chambers and aged in the presence of sunlight. Important atmospheric variables such as temperature, particle size distributions, total solar radiation, and ozone concentration were monitored over the course of the experiment. Concentrations of tetra- and penta-brominated PBDDs and PBDFs were monitored by collecting and analyzing particulate and vapor phase samples. This analysis involved soxhlet extraction, the use of silica, florisor and carbon/celite columns to isolate PBDDs and PBDFs from other compounds and high resolution gas chromatography/high resolution mass spectrometry (Resolving Power = 10,000). Although PBDPEs can exhibit ions that interfere in the analysis of PBDFs, these compounds were not significant interferences in this work.

Results and Discussion. PBDDs and PBDFs partitioned primarily to the particulate phase ([particulate]/[particulate + vapor] = .95-.99). Particulate phase concentrations of PBDDs and PBDFs show no consistent evidence for degradation over periods of up to six hours under realistic atmospheric conditions (see Figure 1). This conclusion is supported both by an examination of the individual data points in view of their experimental variability, and by a statistical analysis of the change in concentration of analyte as a function of incident solar radiation. Though a photolytic production of TBDFs from PBDPEs has been demonstrated both in solution (2) and adsorbed onto filter surfaces, calculations predict that this process is unlikely to significantly influence observed concentrations in our chamber aging experiments. Therefore we believe that a photolytic degradation process for PBDDs and PBDFs, on realistic soot particle surfaces, if occurring, has a half life of at least three hours instead of several minutes as predicted by solution phase experiments.


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Figure 1: Stability of TBDD, TBDF, PeBDD and PeBDF on particulate phase material over 6 hours under warm temperature (39 - 30 C) and bright (1.0 - .22 langleys) sunlight (data is derived from the March 14, 1990 experiment).
A HIGH RESOLUTION MASS SPECTROMETRIC METHOD FOR THE DETERMINATION OF MONOBROMINATED POLYCHLORINATED DIBENZO-P-DIOXINS IN ENVIRONMENTAL SAMPLES

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The analysis of environmental and biological samples for polychlorinated dibenzo-p-dioxins (PCDDs) has received considerable public and scientific attention during the past several decades because of their high toxicity. Recent findings of polybrominated dibenzo-p-dioxins (PBDDs) in some samples have also raised concern about their environmental impact. At the present time, very little is known about mixed bromo/chloro dioxins such as monobromopolychloro dibenzo-p-dioxins (BPCDDs), although structural considerations and biological activity of these compounds indicate that their toxicity is similar to that of PCDDs (ref. 1).

Mass Spectrometry (MS) has been the leading technique used in dioxin analysis, and MS methods for the analysis of PCDDs are well-established. Quantitative analysis of BPCDDs is, however, much more difficult than the methods for PCDDs in that: (1) very few standard compounds of BPCDDs are currently available for calibration and quantitation; (2) the levels of BPCDDs found in samples are a factor of ~30 times lower than those of PCDD counterparts as estimated in an early study (ref. 2); (3) there are many more possible isomers of BPCDDs than that of PCDDs, e.g., maximum possible BPCDD compounds of 198 for tetra- through octa- halogen substitution and 70 for tetra-group, as compared to only 49 PCDD isomers of tetra- through octa- substitution and 22 for tetra-group; (4) mass spectrometric responses of BPCDDs are a factor of 2-5 times lower than that of PCDDs, which requires higher sensitivity of an instrument for the analysis of BPCDDs (ref. 2); (5) the analysis of BPCDDs is more complicated due to the presence of large quantities of chloro analogues, which cannot be separated in cleanup procedures. Under such circumstances, the analytical method must have an extremely high sensitivity and specificity for the quantitative determination of BPCDDs.

In a recent study, a high resolution gas chromatographic/high resolution mass spectrometric method was developed for the determination of BPCDDs in environmental samples. The mass spectrometer was operated at high resolution (>10,000) in the selected ion monitoring mode with magnet switching among tetra- through octa- groups to achieve low or sub-ng sensitivity. Synthesized standard compounds of BPCDDs for tetra- through octa- congener groups were utilized for accurate measurement of the MS relative response factors for the first time. The mean values of RRFs (Table 1) ranged from 0.2 to 0.6 for BPCDDs, as compared to RRFs of close to unity for PCDDs. This finding indicates that previous quantitations for BPCDDs in the literature using PCDDs' RRFs of close to unity underestimated the levels of BPCDDs in the environment. Excellent linear calibration was achieved by using these standard compounds at different concentrations. Correlation coefficients of 0.9988, 0.9980, 0.9984, 0.9990, and 0.9992 were obtained for 2-Br-3,7,8-Cl₂DD, 2-Br-1,3,7,8-Cl₄DD, 2-Br-3,6,7,8,9-Cl₅DD, 1-Br-2,3,6,7,8,9-Cl₆DD, and 1-Br-2,3,4,6,7,8,9-Cl₇DD, respectively. The method detection limit was at low or sub-ng levels for BPCDDs. Accuracy greater than 80% and precision better than 10% were demonstrated with spiked samples. Flyash samples collected from several countries were analyzed for BPCDDs by this method, and substantial amounts of BPCDDs were detected in every sample. Preliminary identification of some BPCDD isomers that were detected in flyash is shown in Figure 1. Isomer specific determination, however, awaits further improvements of chromatographic techniques and availability of BPCDD standard compounds in the future.

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Table 1. Relative Response Factors of BPCDDs to their internal standards of $^{13}$C$_{12}$-PCDDs computed in initial calibration.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CS1</th>
<th>CS2</th>
<th>CS3</th>
<th>CS4</th>
<th>CS5</th>
<th>CS6</th>
<th>Mean RRF</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Br-3,7,8-Cl$_3$DD</td>
<td>0.2061</td>
<td>0.2264</td>
<td>0.1881</td>
<td>0.1908</td>
<td>0.1929</td>
<td>0.1817</td>
<td>0.1977</td>
<td>8.19</td>
</tr>
<tr>
<td>2-Br-1,3,7,8-Cl$_4$DD</td>
<td>0.4843</td>
<td>0.5355</td>
<td>0.3975</td>
<td>0.4504</td>
<td>0.4683</td>
<td>0.4310</td>
<td>0.4611</td>
<td>10.26</td>
</tr>
<tr>
<td>2-Br-3,6,7,8,9-Cl$_5$DD</td>
<td>0.6737</td>
<td>0.6827</td>
<td>0.5071</td>
<td>0.5643</td>
<td>0.5964</td>
<td>0.5528</td>
<td>0.5962</td>
<td>11.70</td>
</tr>
<tr>
<td>1-Br-2,3,6,7,8,9-Cl$_6$DD</td>
<td>0.6303</td>
<td>0.6464</td>
<td>0.5234</td>
<td>0.5743</td>
<td>0.5743</td>
<td>0.5432</td>
<td>0.5820</td>
<td>8.26</td>
</tr>
<tr>
<td>1-Br-2,3,4,6,7,8,9-Cl$_7$DD</td>
<td>0.2853</td>
<td>0.3157</td>
<td>0.2435</td>
<td>0.2825</td>
<td>0.2591</td>
<td>0.2717</td>
<td>0.2763</td>
<td>8.96</td>
</tr>
</tbody>
</table>

aCalibration Standard solutions.

Figure 1. Chromatographic traces of BPCDDs.

Reference
APPLICATIONS OF MASS SPECTROMETRY TO THE IDENTIFICATION OF MUTAGENIC COMPOUNDS PRODUCED BY MONOCHLORAMINATION OFFULVIC ACID

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INTRODUCTION. Chlorination of drinking water that contains natural organics leads to the formation of mutagenic nonvolatile chlorinated compounds. These compounds include propenals, propanones, oxo-butenoic acids and hydroxy furanones. In view of the human health hazards posed by chlorine by-products, monochloramine has gained importance as a possible alternative disinfectant. However, information regarding the potential human health hazards associated with the use of monochloramine is very limited. Although several researchers have identified mutagenicity in monochloraminated humic and drinking waters, few have been able to identify products leading to this mutagenicity. The objective of this research was thus to identify compounds in the mutagenic extracts produced by the reaction of monochloramine and fulvic acid. Fulvic acid was chosen as a model compound since it represents approximately one half of the organics naturally present in drinking water sources.

METHODS. Monochloramine was prepared fresh by reacting ammonium chloride and sodium hypochlorite in a 3:1 molar ratio at pH 8.0. Monochloramine was reacted with fulvic acid at the desired Cl:C molar ratio at pH 8.0 over a period of 96 hours. Chlorination of fulvic acid was performed by reacting sodium hypochlorite with fulvic acid at a molar Cl:C ratio of 1:5. Extraction of the sample was by liquid-liquid extraction with diethyl ether. Extractions were performed at sample pH 2.0. The mutagenicity of the extracts was assayed in accordance with the standard plate incorporation assay by Maron and Ames (1983).

Results of preliminary experiments suggested that the mutagenic compounds were acidic and polar. We therefore derivatized mutagenic extracts with boron trifluoride (BF$_3$) in methanol because it methylates hydroxyl-, dihydroxyl-, carboxyl-, dicarboxyl- and aldehydic functional groups. Diethyl ether extracts were taken to dryness under a stream of N$_2$ gas and then redissolved in 250μL of methanol containing 14% (v/v) BF$_3$. The mixture was allowed to react for 12 hours in a 70°C mineral oil bath. The mixture was subsequently neutralized with 3mL of 2% (v/v) NaHCO$_3$, extracted twice with 250mL of hexane and concentrated under a stream of N$_2$ gas.

The derivatized extracts were then analyzed by using high resolution gas chromatography / mass spectrometry (HRGC/MS). The analyses were performed on a Hewlett Packard 5890 gas chromatograph interfaced with a VG70-25SEQ mass spectrometer (resolving power = 1,000 or 10,000; 10% valley definition). All experiments were conducted using a DB-5, 30-m-length, 0.25-mm-i.d., 0.25μm film fused silica capillary column. Experiments performed by full-scan mode were conducted using a GC temperature program of 50°C for 1 minute followed by a temperature rise of 2.5°C/min to 150°C and 5°C/min to 300°C. A temperature program of 50°C for 1 minute and a rise of 10°C/min to 300°C was used in analyses conducted by selected ion monitoring (SIM).

Full-scan mass spectra were acquired by using electron-ionization (EI) at an electron energy of 70eV, 200μamp trap current, 250°C source temperature and resolving power of 1,000. The magnet was scanned at 500-50 amu at 1 second/decade. Quantifications were performed by measuring the relative response of a specific ion of each of the compounds to the response of the m/z 334 ion of the internal standard, decaffluorobiphenyl (10ng/μL).

Selected ion monitoring analyses were performed under similar instrumental conditions as full-scan analyses except that a resolving power of 10,000 was used. The internal standard used was $^{13}$C$_6$ benzoic acid (50pg/μL) and the recovery standard for quantification of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) was anthracene d$_{10}$ (25pg/μL). MX quantification was based on a four point calibration curve constructed from the analysis of standard solutions that contained 50, 100, 250 or 500pg/μL of MX, and the internal and recovery standards. Semi-quantification of E-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid (EMX), E-2-chloro-3(dichloromethyl)-butenedioic acid (ox-EMX) and mucoochloric acid was performed based on a relative response factor obtained from the analysis of a single standard solution of each analyte that containing 50pg/μL $^{13}$C$_6$ benzoic acid.
RESULTS AND DISCUSSION. Analyses in the GC/MS full-scan mode resulted in the analyses of forty-five short chain (C₅ - C₁₀) saturated and unsaturated aliphatic chlorinated compounds. The majority of these compounds were identified on the basis of their electron-ionization mass spectrum. The identities of di- and trichloroacetic acids, dichlorosuccinic acid and ox-EMX were confirmed by performing analyses on authentic standards. The products identified are all esters derived from the methylation of chlorinated alcohols, chlorinated aldehydes (which may also be dialcohols due to the nature of the derivatization) and chlorinated acids. About a third of the compounds identified, mostly acids, have previously been observed as chlorination by-products. Unique to this work are several alcohols which may be characteristic of monochloramination products. Of particular interest are the several alkenoic acids identified which may be of toxicological significance because of their structural similarity to the mutagenic open and oxidized forms of the potent mutagen, MX.

Selected ion monitoring analyses resulted in the identification and quantification of MX, and semi-quantification of EMX, ox-EMX and mucoccloric acid in monochloramination as well as in chlorination extracts. Chlorination produced at least twice as much of each of these compounds as monochloramination. MX, EMX and ox-EMX were found to contribute 11%, 26% and 2%, respectively to the mutagenic activity of the monochloramination extract.

Table 1. Examples of Chlorinated Alcohols and Aldehydes Identified by GC/MS

<table>
<thead>
<tr>
<th>Compound Type</th>
<th>Structure Identified</th>
<th>Possible Precursor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td>CH₂Cl-C(OCH₃)₂-CH₃</td>
<td>3-chloro-2,2-propanediol</td>
</tr>
<tr>
<td></td>
<td>CH₂Cl-CCl(OCH₃)-CH₂CH₃</td>
<td>1,2-dichloro-2-butanol</td>
</tr>
<tr>
<td>Dials or</td>
<td>CH₂Cl-CH(OCH₃)₂</td>
<td>2-chloro-1,1-ethanediol</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>CHCl₂-CH₂-CH(OCH₃)₂</td>
<td>or chloroacetalddehyde</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3,3-dichloro-1,1-propanediol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or 3,3-dichloropropanal</td>
</tr>
<tr>
<td>Acids</td>
<td>COOCH₃-CCl₂-COOCH₃</td>
<td>dichloropropenediol acid</td>
</tr>
<tr>
<td></td>
<td>CH(OCH₃)₂-CCl-CCl-(CH₂)₄-COOCH₃</td>
<td>6,7-dichloro-8,8-hydroxyoctanoic acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or 6,7-dichloro-8-oxo-octanoic acid</td>
</tr>
</tbody>
</table>

CONCLUSIONS. Forty-eight compounds were identified in the mutagenic extracts of monochloraminated fulvic acid by HRGC/MS analyses. Of these, forty-four are novel to this work. The potent mutagen, MX and its tautomers EMX, ox-EMX were found to contribute 39% to the mutagenic activity of the monochloramination extract. The remainder of the activity may be explained by the several alkenoic acids identified as they are structurally similar to the open and oxidized forms of MX.

REFERENCES.
Computer Aided Interpretation of Low Energy MS/MS Mass Spectra of Peptides

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Joe X. Zhou
Finnigan MAT
San Jose, CA 95134

The development of tandem mass spectrometry for the determination of internal amino acid sequences in proteins will make a significant impact on the field of structural biology. The ability to directly analyze complex mixtures of peptides derived from proteolytic digests of proteins will increase the rate at which amino acid sequences can be determined. Limited structural information obtained from tandem mass spectrometry experiments on proteins can be used to design and synthesize deoxynucleotide probes for cloning the corresponding gene. An integrated high sensitivity, high throughput protein sequencing strategy based on tandem mass spectrometry would be ideal for the characterization of a large array of proteins such as those involved in transcriptional regulation. However, the amount of MS/MS sequence data derived from such a large scale analysis would require many hours for successful interpretation. Real time or near real time interpretation of the data would make such an approach a powerful tool for the molecular dissection of key biological processes.

The development of computer algorithms for the interpretation of mass spectral data has been a long standing goal (1-3). Early attempts at automated interpretation were hampered by insufficient computational power to provide solutions in a timely manner. This was particularly true for the determination of peptide amino acid sequences where a 10 residue peptide could have $10^{20}$ possible sequences. Computer technology has been evolving at a phenomenal rate with computer processor speeds doubling every 12-15 months. A consequence of these rapid technological advances is that computational approaches should no longer be constrained by processing speed.

We have developed two approaches for the interpretation of low energy MS/MS spectra of peptides. The first approach was designed for the interpretation of the singly charged mass spectra produced by MS/MS of peptides ionized by LSIMS. Peptides analyzed by this method are rich in fragment ions of type b- and y"-. The second computational approach has been optimized for the interpretation of mass spectra produced by MS/MS analysis of doubly charged peptide ions from electrospray ionization. In this type of mass spectrum y"- type ions are frequently the most abundant. Peptides derived from tryptic digests of proteins will consist predominately of doubly charged peptide ions. The fragmentation patterns of doubly charged these peptides are more consistent and easier to interpret than those of higher charge states.

LSIMS mass spectra were recorded as previously described on a Finnigan MAT (San Jose, CA) TSQ 700 triple quadrupole mass spectrometer equipped with a 20 keV conversion dynode and 40 keV cesium ion gun (4). Electrospray ionization mass spectra were recorded on the same mass spectrometer equipped with an electrospray ionization source. The molecular weights of peptides were recorded by scanning Q3 at a rate of 400 amu/sec over a mass range of 400 to 1600 throughout the HPLC gradient. Sequence analysis of peptides was performed during a second HPLC analysis by selecting the parent ion with a 6 amu wide window in Q1 and passing the ions into the collision cell which was filled with argon to a pressure of 5 mtorr. Collision energies were on the order of 20 to 50 eV. The fragment ions produced in Q2 were transmitted to Q3, which was scanned at 500 amu/sec over a mass range from 50 amu to the parent mass to record the fragment ion mass to charge ratios.

Under typical operating conditions a 3-6 dalton window in Q1 is used to select the parent ion.
This window will also pass the (M+H)\(^+\) \text{C}^{13}\text{ containing ions giving a cluster appearance to the parent ion region and the fragment ions. Fragment ions produced under low energy multiple collision conditions of the triple quadrupole are dominated by } b^-\text{ and } y^-\text{ type ions. Neutral losses of water and ammonia from } b\text{ ions are frequently observed and } a\text{ ions (b - 28) are also prevalent. Clusters of ions 17/18 and 28 daltons below another ion are frequently diagnostic for } b\text{ ions. } y^-\text{ ions can also be accompanied by neutral losses of water and ammonia. The MS/MS spectra of doubly charged peptides are often predominated by } y^-\text{ type ions and the neutral losses are of less intensity than observed in the singly charged MS/MS spectra produced by LSIMS. These sets of fragment ions are the major fragmentation pathways for peptides under low energy collisions and are the primary ions used for the identification of amino acid residues.}

The strategy used in the interpretation of LSIMS MS/MS spectra is to sequentially subtract the weights of each of the amino acids from the (M+H)\(^+\) ion and compare the predicted fragment ion with the mass spectrum. A score is generated on the basis of the intensity of the } b^-, b^-17, a^-\text{ and } y^-\text{ ions. The scores are then sorted to generate a list of partial sequences for the next subtraction cycle. If proline or histidine are found in the sequence the program checks for } b_ny^-n^-\text{ type ion series. The cycles continue on a particular amino acid sequence until subtraction of the mass of an amino acid leaves a difference between the predicted and observed (M+H)\(^+\) of less than 0.50 but more than -0.5. This sequence is saved for final scoring.}

A final score is calculated from three factors: the number of peaks in the mass spectrum (free acid and methyl ester, if used) accounted for in the mass spectrum, the average intensities of predicted fragment ions, and the continuity of sequence ion occurrences within a given fragmentation scheme. Any known sequence information can be provided to the program such as the presence or absence of amino acids, the extension directions (N to C, or C to N) and any known residues. The program also allows graphical analysis of the results obtained by comparison of the predicted fragments for a sequence with the mass spectrum.

A different computational approach was used for the interpretation of ESI MS/MS spectra. The mass spectral data is smoothed and ions below a set threshold are eliminated from the spectrum. The spectrum is then divided into sections of approximately 50 daltons each. The fragment ion of the greatest intensity in each section is identified and the boundaries are shifted to those ions classified as } y^-\text{ type ions. Amino acid sequences are then determined for the molecular weight differences between boundaries. Where continuous } y^-\text{ type ions have been found the differences between the ions will correspond to the weight of a single amino acid. In cases where the differences between boundaries correspond to two of three amino acids, all combinations corresponding to the weight difference are determined and their predicted fragment ions calculated. An average score based on predicted fragment ion intensity is calculated and the sequences sorted. The time required to interpret a mass spectrum is negligible. For each boundary determination the user can reject the determined boundary and choose another. Only the mass spectrum corresponding to the underivatized peptide is required for interpretation.}

In the methods described above no attempt has been made to differentiate Leu and Ile and Gln and Lys are differentiated on the basis of enzyme specificity or the chemical reactivity of the e-amino group of Lys. Computer aided interpretation of MS/MS spectra represents a promising approach for increasing the throughput of protein sequencing by tandem mass spectrometry.

MASS SPECTROMETRY IN CONTEXT. SOLVING STRUCTURAL PROBLEMS IN A NETWORKED MULTI-VENDOR ENVIRONMENT.

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To function effectively and efficiently, within a large organisation, an analytical department offering a wide range of spectral techniques has to overcome numerous problems. These can be broadly categorised as follows:— management of sample throughput, handling data acquired from different spectral techniques with incompatible computer systems and data formats, review and storage of data is often restricted to 100% OEM systems, multiple paper transactions and only a few specialists capable of retrieval of data from multi-disciplined areas.

One solution to these problems is to base a system in the PC environment and utilize a PC local area network (LAN). Thereby using an operating system and software familiar to both Chemist’s and Analyst’s, whilst also making use of bulk storage devices such as optical disks possible. The objectives in implementing the system were to remove intra-company boundaries and enable the ‘pooling’ of analytical information for retrieval from any node on the network.

A Novell PC LAN was used along with a chemical structure database package to achieve the proposals outlined above. In the mass spectrometry laboratory, an EM4105 terminal emulator was used which provides a screen capture to file facility. The captured screen image is a mass spectrum which would normally be hardcopied and returned to the Chemist with an attached text report. The terminal emulation package can be setup to direct captured files automatically to shared networked areas specific to the instrument on which the data was acquired (see Figure 1).

This can also be indexed by date and so provides a reliable method of archiving.

![Diagram](image_url)

**Figure 1. File capture and management over the PC LAN**
The Chemist makes a request for analysis by entering information about the sample in the database. The Spectroscopist then identifies it as a new entry in the database and transfers the information as ASCII data to the spectrometer. After analysis, the spectrum/spectra is captured and converted to a metafile. The spectra from the sample, analytical conditions, date completed, files assigned for each spectroscopic technique and a brief text report can then be stored in the database for retrieval from any node on the PC LAN. The results can be displayed in a number of formats determined by which fields, in the database, are displayed in the report form, an example is shown in Figure 2.

<table>
<thead>
<tr>
<th>CHEMIST'S INDEX NUMBER</th>
<th>SPECTROSCOPY REFERENCE NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHEMIST REFERENCE</td>
<td>RELATED FILES</td>
</tr>
<tr>
<td>DEPARTMENT</td>
<td>MS METHOD</td>
</tr>
<tr>
<td>MOLECULAR FORMULA</td>
<td>ANALYST</td>
</tr>
<tr>
<td>MOLECULAR WEIGHT</td>
<td>DATE COMPLETED</td>
</tr>
</tbody>
</table>

The sample was analysed using Fast Atom Bombardment (FAB) MS. The molecular ion obtained is consistent with the proposed chemical structure.

The system described has been implemented and is in daily use. The likelihood of human transcription error has been greatly reduced. Two criteria of the system give the Spectroscopist more time to improve the quality of data produced:—no time is required for filing paper copies—all data and text report summaries are stored electronically together and secondly the responsibility for ‘backing up’ the File Server lies with personnel who maintain the PC LAN and not Spectroscopist’s. One negative aspect is that if there is a problem with the PC LAN, then the system grinds to a halt.

The implementation of this system has been met with enthusiasm by both Chemist’s and Spectroscopist’s. Its reliability is very good and in the future will enable us to seek a higher degree of integration allowing a higher degree of automation to follow.
COMPUTER-AIDED ASSEMBLY OF COMPLETE PROTEIN SEQUENCES FROM
THE PROBABLE (CID-MS DERIVED) SEQUENCES OF PROTEOLYTIC PEPTIDES.
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INTRODUCTION:
The algorithm SEQPEP\(^1\), predicts sequences from the characteristic
fragments ions observed in the CID spectrum of the peptide, then ranks them
from a score of 1.0 (maximum) to 0.0 (minimum) by decreasing fit with the
input CID data. For example a score of 0.827 indicates that the sequence
accounts for 82.7% of the sum of all ion abundances of the input spectrum.
However, the highest ranking sequence is not necessarily the correct one,
mainly due to the possibility that certain combinations of one to four
amino acids have the same residue mass, i.e. are isobaric (R=156=GV). If
there is a peak in the spectrum that happens to fit a potential fragment
of the incorrect combination, than that sequence will have a higher score
than the correct one. In such instances it is then not possible to arrive
at the correct protein structure by using the highest scoring sequence of
each peptide.

The redundancy of information represented by the CID spectra of
peptides generated by two or more proteases with differing specificities
can be used to recognize the correct sequence even if it does not have the
highest SEQPEP score. This is because it is unlikely that the same
ambiguity exists in the spectra of peptides derived from very different
cleavage sites but partly representing the same region of the protein. The
corrected peptide sequences must then be assembled to the final protein
structure.

The process described above can be accomplished manually, but is
tedious and time consuming, hence is best done by using a computer. We
report here an algorithm, COMBSEQ, that does it automatically until the
final stage which is interactive to allow human inspection of ambiguous
situations (which are flagged). As an example we are using the output of
SEQPEP for the CID spectra of 30 peptides which were used to determine the
complete structure of glutaredoxin (Grx) isolated from rabbit bone marrow
(RBM) which had been deduced by manual correlation of the data\(^2\). The
peptides were produced by digestion of the protein with trypsin (11
peptides), Chymotrypsin (9), Endo Glu-C (8) and thermolysin treatment of
some tryptic peptides\(^2\).

PROCEDURE AND DISCUSSION:
The approach to the assembling of the peptides to the final protein
structure involves the following steps:

(a) First assume that the highest ranking sequence is the
    correct one.
(b) Use them to extend by overlaps.
(c) Obtain full overlap by substituting highest ranking sequence
    which does not overlap with a lower ranking sequence that does.
(d) Flag amino acid positions which may be ambiguous and
    needs human inspection and decisions.

The input data for COMBSEQ are the top scoring 20 sequence generated
by SEQPEP for each CID spectrum of 30 peptides produced by the above
described digests of RBM-Grx\(^2\).

COMBSEQ begins with the assumption that the highest scoring sequence
is the correct one, if it is at least compatible with the corresponding
enzyme specificity. This assumption does not hold for the C-terminal
peptide and in the final sequence the C-terminal amino acid should,
therefore, be scrutinized carefully. Each of the 30 peptides are searched
for greater than or equal to 2 amino acid overlaps with the 29 other
peptides. A set of 16 peptides resulted, of which 10 were unmodified and
the remaining 6 were overlapping combination of the other 20. Since the
molecular weights of the 20 highest scoring sequences for each peptide must
be identical, the ambiguities among the various possibilities are generally
due to isobaric combinations (e.g., AG=GA=Q=K or MA=AM=DS=SD or PSN=GL etc.). At this point COMBSEQ simply checks whether the mass of any of these peptides is a component (and that at least half the sequence matches) of any of the other 15. This isobaric match now results in 7 partial sequences.

At this point one has to remember that the sequences used even at this stage are not necessarily correct. The regions of ambiguity are revealed by the apparent lack of overlap between the C-terminal of one and N-terminal of another of the 7 partial sequences generated up to this point. If any of them indeed overlap then C-terminal of one must be isobaric with the N-terminal of the other. By matching the mass of all the C-termini (up to 4 amino acids) with that of all the other N-termini (also up to 4 amino acids), COMBSEQ recognizes that there is a pair which combine by overlapping (isobaric N-terminal & C-terminal match) as —CR=DSN--. This is confirmed by checking the input data which contain a number of sequences of lower scores beginning with CR--. One of them overlaps with other peptides and is therefore the correct one. A second such ambiguity --GNQX=GELL-- is resolved in the same way. These matches further reduces the total number of partial sequences to 5.

Two of these five partial sequences contain the same 18 amino acid sequence. The repeated occurrence of such a long sequence is very unlikely. Therefore COMBSEQ separates them in two sets of 5 partial sequences so that none of the sets have any common units.

For the final assembly of the two sets of 5 partial sequences COMBSEQ uses the molecular weights of larger peptides generated with a very specific enzyme but not sequenced by CID. In the case of RBM-Grx molecular weights of four endo Glu-C peptides were [(M+H)*]=1044.71, 1486.95, 3223.72 & 3953.06. Twelve of the possible 144 complete protein sequences were found matching at least three of these (M+H)* ions, but only one matched all the four (M+H)* ions and thus is most closely correct.

Up to this step the entire process is automatic. Now COMBSEQ goes into an interactive mode and displays for each possible sequence all positions where alternatives exist (by comparing it with all the 20 highest scoring sequences of SEQPEP for the 30 CID spectra), in a format that facilitates human intervention and inspection.

The ambiguous positions are corrected or reconfirmed from the peptide specificities and/or number of occurrence found for the particular position. For example; a sequence MDAA (positions 79-82) comes from one first ranked M, 1D, 3A and 2A, but there are 10 lower ranked sequences that have DCX and one DCI in this position. Since DCX or DCI (X=I or L) is isobaric with MDAA we replace MDAA with DCI after verifying I from d and w ions. Once all the ambiguous positions have been clarified and corrected, the final answer, the complete primary structure of protein, is obtained.

CONCLUSION:
An algorithm, COMBSEQ, has been written which assembles amino acid sequences of proteolytic peptides, derived from their CID spectra, to the complete primary structure of protein. The program is written in VAX-FORTRAN and requires 1-10 minutes to find the final complete sequence, reducing the time and effort to do manually.

REFERENCES:

ACKNOWLEDGEMENTS:
The authors are indebted to R.S. Johnson who determined the CID spectra and J. E. Biller for his help with the VAX-workstation. This work was supported by a grant from the National Institute of Health.

Over the course the last five years our laboratory has strived to institute a network system of mass spectrometry data systems. We have previously reported on our efforts to archive data from our VG 11-250J and Nermag Sider systems has been previously reported. This system allowed transfer of data between acquisition computers and an archival computer. Using a local area network (LAN), appropriate remote terminals can access software and data on each acquisition computer. In April 1990, three Kratos Mach 3 data workstations were acquired and placed on the network. These are being used to process data from two VG mass spectrometers as well as a Concept 1S mass spectrometer. Future plans are to process data from other instruments in the laboratory as well as data received from other laboratories. The installation of this workstation data system has been named a "universal" mass spectrometry laboratory system. This unwieldy title is used to distinguish it from the acquisition data systems which process data from only one mass spectrometer.

Having this "universal" system in the laboratory has provided several new capabilities to the laboratory. First, mass spectral data from different instruments can be processed on the same system without the need to go from one terminal to another. The data can also be archived to and retrieved from one system. Over several years, this means that the data from retired data systems can be accessed which is important under GLP guidelines and when plotting mass spectra for publication. Also, the software of the Mach 3 system can process data more efficiently than the older data systems. Analysis time for long GC/MS runs has been halved and the analysis of multichannel analysis (MCA) mass spectra of peptides has been reduced by two thirds.

Another advantage of the universal data system concept is the ability to analyze data produced at diverse geographical sites. The Wellcome Research Laboratories are situated in three separate buildings approximately one mile apart with mass spectrometers located in two of those sites. The need to analyze data at other sites can be achieved by use of the network and the Mach 3 universal system. Data produced in one building need not be reproduced in hardcopy form prior to discussion with chemists but can be shipped to the chemists location for viewing. This procedure saves time on the part of laboratory personnel who no longer have to plot or reprint data for the chemists. Also, BW sends out approximately 4200 samples per year for CI analysis to an outside facility. Presently, the data for each sample are returned as hard copy output via Federal Express which takes three days turnaround per sample set with samples being sent each working day. Using this procedure, no spectral enhancement can be done to elucidate small peaks nor can new mass chromatograms be plotted to verify coelution of ions to check for impurities. A new procedure is being instituted whereby the data are transmitted via phone-link to the Mach 3 system, converted, and plotted at BW. Such data can be reanalyzed if necessary. Also, the analysis of these data by the chemists without the intervention of mass spectrometry laboratory personnel is possible and is being considered.

As the laboratory grows we find that this system may be able to reduce the costs of purchasing new equipment and the number of extra terminals required in the laboratory. When new machines have been purchased in the past, extra terminals and sometimes data systems have been purchased with the mass spectrometer to facilitate data analysis. This is not necessary if the data can be transferred to the
universal system for analysis.

While the advantages listed above have made the universal system an attractive and valuable addition to our laboratory, we have encountered problems in its implementation. Most of these have had to do with the incompatibility of data formats from one machine to another. For instance, there is as yet no standard file structure for mass spectral data except for mass/intensity data, no provisions have been made for MCA data, MS/MS data, or single ion recorded data. Therefore conversion programs have to be written for each type of data from each machine. This has turned out to be a time consuming and costly procedure. We are in the process of writing conversion programs for the Nermag Sidar system at the present time. Other conversion programs for our VG equipment and Finnigan data systems were provided with the Mach 3 purchase. However, even these have required modification due to differences in the algorithms used for data acquisition and in the calibration procedures used by the various vendors. In order to analyze the converted data, changes had to be made in scan laws and/or scan functions to produce mass spectra that can be processed by the Mach 3 system. This has been accomplished for high and low resolution MCA data, but has not been implemented for linked scan data.

In regard to data conversion problems, mass spectrometer users must be trained to recognize that problems may exist in the use of a "universal" system and attempt to overcome them. The same training must be done for chemists who analyze their own data. We have found however that this extra effort is rewarded by the tremendous increase in speed and efficiency afforded by newer systems over systems five to ten years old.

Cost can be a problem in the purchase of a "universal" data system. These systems at present require data workstations which contain both computing power and resident software which requires both expensive hardware and expensive software licenses respectively (~$10,000/workstation). This is much more than the cost of two or three extra terminals per mass spectrometer for a conventional system (~$800/terminal) where no software license is needed.

The cost of a "universal" system can be lowered and some of its disadvantages can be overcome in the future by several steps. Faster and more powerful processors should become available as witnessed over the last twenty years. If these systems are adopted by the mass spectrometer vendors, then the cost for an equivalent amount computing power should be lowered. Also, the need for purchasing several workstations with their accompanying expensive software license can be eliminated by the institution of windowing in the host mass spectrometer data system environment. One fast, powerful processor could be used to analyze the data from any machine. This main processor could then be accessed from X-window terminals or other data systems running X-window software with all data manipulations being done on the main processor and viewed on the X-window device. This process is now being developed by several computer manufacturers and should be fully utilized by the mass spectrometer vendors. Finally, the vendors should agree on common data formats for all types of mass spectrometry data, and they should provide programs for conversion of their data to and from the standard file formats. With these changes the implementation of "universal" laboratory systems could become attractive to more laboratories.
A computer program called MacProMass has been written to assist protein chemists and mass spectrometrists in utilizing mass spectral data for peptide and protein structure analysis. The program runs on any Macintosh computer with 1 Mbyte of memory and a hard disk. Full advantage is taken of the Macintosh graphical user interface. Sequence data can be entered manually, pasted in from the clipboard, or imported from a text file. The main display is arranged such that any portion of a sequence in length up to 2000 residues can be readily located. For a user-selected portion of the sequence, values are calculated for monoisotopic and average mass, elemental composition, isoelectric point, HPLC index, surface free energy, and amino acid composition. Search routines are provided to locate portions of a sequence with a given mass, sequence, or amino acid composition. Mass values are calculated either for the protonated molecule or the molecule minus a proton. The m/z value can also be calculated for any number of charges. The theoretical isotope pattern can be calculated for any portion of the sequence.

The twenty common amino acids which are coded by DNA are permanently designated by the standard single letter code. The remaining six letters in the alphabet can be programmed by the user to have any composition which uses the elements C, H, N, O, S, P, F, Cl, Br, and I. Additionally, both the C-terminal and N-terminal groups can be programmed. A separate section of the program provides the means to easily calculate mass values for complicated multichain structures with multiple inter- and intra-chain linkages such as disulfide bonds. These connections are made using a clever graphical display that allows the user to simply draw in a bond of a selected type using the mouse. A list of several possible bond types is provided or the user may define his own. Thus, MacProMass can model almost any protein structure, real or imagined.

A number of routines are provided that greatly simplify data analysis. Mass spectral fragments can be calculated for any linear peptide up to 100 residues in length. The output can be in terms of a table arranged according to ion series or by increasing mass. The user can choose to use either the Roepstorff or Biemann nomenclature to designate ion series which may be numbered from either the N- or C-terminus. The user can select which ion series to include in the table choosing either the positive or negative ion mode. Default values can be set so that the user can customize the program to his particular needs. The fragment tables can be searched to identify particular ions in a spectrum either individually or in a group. Those values identified in a search are identified with bold face type. There is also a routine that will calculate possible fragments resulting from two bond cleavages (so called...
internal fragments). Values in the fragment tables can be adjusted to accommodate up to four charges.

The enzyme digest routine can be used to calculate the mass of peptides that result from enzymatic or chemical cleavage of a protein. The common enzyme and chemical cleavages are supported directly, and the user can create his own by defining the specificity of the cleaving reagent. The resulting list of peptides can be arranged according to position in the original sequence, mass, isoelectric point, HPLC index, or surface free energy value. For structures that contain inter- or intra-chain bonds, mass values are calculated for the linked fragments. For proteins that have disulfide bonds that are undefined, a routine is provided that finds all of the possible linked peptides that correspond to a given mass. This routine is very useful for determining the location of disulfide bonds. A second routine is provided to assist in analysis of protein variants that result from the substitution of a single amino acid residue. This routine systematically considers all of the single amino acid substitutions which will account for the mass difference between an observed mass in an enzyme digest mixture and those calculated based on the expected sequence.

A section called Hydrophobicity which is largely unrelated to mass spectrometry has been included as a convenience to protein chemists who do secondary structure analysis from primary sequence data. In addition to the Hopp & Woods, Kyte and Doolittle, and Engleman, Steitz and Goldman hydrophobicity scales, there are scales for antigenicity, chain flexibility and Chou Fasman secondary structure analysis.

In conclusion, MacProMass is a versatile, multifunctional, user friendly program that frees researchers from many of the tedious calculations involved in doing mass spectrometry on peptides and proteins.
Accurate Molecular Weight Determination from Near-symmetrical, Unsymmetrical, Broad or Inadequately Resolved Peaks

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Ionspray quadrupole mass spectrometer has been used to measure molecular weight (MW) of proteins. To assist the accuracy of MW determination, a polynomial curve fitting method was developed in the data analysis [1]. The method was applied successfully to bovine serum albumin, of which MW was estimated as 66,431.5 ± 1.3 Da by this method. Lately the sequence MW of the protein was corrected to 66,450.3 Da by discovering missing Tyr-156 [2].

API III ionspray mass spectrometer (SCIEX) used in this study was calibrated using polypropylene glycol. The calibration was corrected further using eq. 2 described below.

A polynomial curve fitting method is a modification of Gaussian curve fitting. Instead of Gaussian curve (eq. 1), natural logarithm of the curve (eq. 2) is used for the fitting.

\[ I = I_0 \exp\left\{-\frac{(x-H)^2}{2\sigma^2}\right\} \]  
\[ \ln(I) = A(x - \mu)^2 + B \]  

where \( x \) is the m/z value at each data point, \( \mu \) is m/z at the peak apex, \( \sigma \) is the peak width, and \( I_0/\sqrt{2\pi\sigma} \) is the peak height at the apex. The eq. 1 is converted to eq. 2, where the fitting is weighted by the logarithm of the intensity in eq. 2 being close to uniform fitting. Since the fitting with eq. 2 is sensitive to the peak shape, the fitting of commonly observed peak shapes, near-symmetrical, unsymmetrical and broad peaks, was examined.

Near-symmetrical monoisotope peaks of singly charged synthetic peptides were analyzed by fitting the top portion (> 50-70% of the maximum intensity) of the peak using eq. 2 (Fig. 1). The experiments were carried out with various synthetic peptides (MW 500 - 2000). The average MW error from 48 experiments was -2 ppm with a standard deviation of 46 ppm. The results suggest that average MW of many measurements is fairly accurate, although a single data has a large error. This is advantageous for MW determination of proteins, which provides average MW of many peaks in a single measurement.

Very often the peaks are not symmetrical in a mass spectrum. The applicability of eq. 2 to unsymmetrical peaks was examined by fitting nearly entire peak of doubly charged synthetic peptides (MW 800 - 2,500) (Fig. 2). The unsymmetry of these peaks is due to the unresolved isotope profile. The degree of the unsymmetry varies due to the different contribution of the isotope peaks in these peptides. The average MW error from 40 experiments was 22 ± 60 ppm. Although there is a systematic error, which overestimates the contribution of isotope profile, the average MW is fairly accurate.

Monoclonal antibodies (MW ~ 150,000) have shown broad peaks due to the heterogeneous glycosylation. Eq. 2 applied to these broad peaks of three monoclonal antibodies by fitting the top portion (> 50-70% of the maximum intensity) of the peaks. Average MW of monoclonal antibodies against α-1-antitrypsin and α-1-acid glycoprotein were determined as 148,484 ± 4 Da (precision 27 ppm) (Fig. 3) and 149,599 ± 12 Da (precision 80 ppm), respectively. Interestingly, monoclonal antibody against β-galactosidase showed two components with average MW of 150,544 ± 10 Da (precision 66 ppm) and 151,496 ± 17 Da (precision 112 ppm). Thus a high precision can be obtained for broad peaks of high molecular weight proteins using the polynomial curve fitting.

When peaks are not resolved to at least 50%, the overlap of the peaks shifts the peak apexes, which makes the experimental MW deviate from the true value. The peak shift was simulated using two Gaussian curves resolved partially. A correction table of the peak shift was obtained as a function of the resolution and the relative intensity of the peaks (Fig. 4). The method was applied to bovine serum apotransferrin (MW 78 kDa). The peak has an unresolved peak, which has additional 87 Da estimated from peak apex. With the peak shift correction, the MW difference was restored to 97, which indicates sulfate or phosphate adduct.

Quadrupole mass spectrometer has a low resolution, and near-symmetrical, unsymmetrical, broad and partially resolved peaks are observed commonly in the spectra. However, a simple data analysis by the polynomial curve fitting or the correction table gives accurate MW determination of proteins.

References:
Figure 1. Polynomial curve fitting of near-symmetrical peak. Raw data and curve fitting are shown with solid and dashed lines, respectively. Curve fitting was performed in logarithm scale in Fig. 1B. MWobs. of a synthetic peptide (MWcalc. 816.559) is 816.505 with an error of 0.054 (66 ppm).

Figure 2. Polynomial curve fitting of unsymmetrical peak. Raw data and curve fitting are shown with solid and dashed lines, respectively. Curve fitting was performed in logarithm scale in Fig. 2B. MWobs. of a synthetic peptide (MWcalc. 817.012) is 817.030 with an error of 0.018 (22 ppm).

Figure 3. Polynomial curve fitting of broad peaks. MW of monoclonal antibody against α1-antitrypsin was observed as 148,484 ± 4 Da (precision 27 ppm) by the curve fitting of five peaks with asterisk.

Figure 4. Apex shift of inadequately resolved peaks as a function of resolution and relative peak intensity. Total intensity of the two partially resolved peaks is normalized to 1.0. Resolution is defined as an intensity ratio between a peak apex and valley.
It has become apparent that computer manipulation of collision induced dissociation (CID) mass spectral data is required if the potential gain in peptide sequencing efficiency using mass spectrometry is to be realized. Two computer programs have been developed at the U of Washington - LUTEFISK and LEFSA - and are described here. The former program is designed to assist in the interpretation of low energy CID data of singly or multiply charged precursors; the latter incorporates both CID and gas phase sequence data in the structure determinations. LUTEFISK is conceptually similar to earlier algorithms in that partial sequences encompassing the C-terminus that account for the greatest number of ions present in a spectrum are extended until the molecular weight of the peptide is reached. Once this subsequecing procedure is finished, the list of completed sequences are assigned scores and ranked based on the fraction of product ion current that can be accounted for as either sequence-specific or non-sequence-specific ions. Of course, LUTEFISK concerns itself only with those ions associated with low energy collisions - predominantly b\textsubscript{n}, y\textsubscript{n}, and an occasional a\textsubscript{n}, all of which can lose 17 or 18 amu, or have multiple charges. It has also been necessary to allow for the absence of fragmentation between adjacent amino acids by allowing subsequences to be extended two amino acids at a time. Although the initial results depicted in Table I have shown that CID spectra of smaller peptides (MW < 2000) with few charges are readily interpreted, the program frequently produces a number of other sequences that account for the fragment ions equally well. For instance, the correct sequence for entry 7 of Table I (AQDFVQW) was assigned the third highest score, and the number in parenthesis indicates that there were eleven sequences of equal or greater score (i.e., there were many sequences with identical scores). Furthermore, fragmentations between adjacent amino acids may not always be observed in these spectra in which case LUTEFISK will identify the correct pair of amino acids; however, the sequence of these two amino acids remains uncertain.

Such problems can be resolved in a number of ways - manual Edman degradation followed by mass spectrometry, derivatization, assembly of probable sequences from overlapping peptides, etc. LEFSA is a program that incorporates both gas phase sequence data of peptide mixtures and CID spectra of those peptides in an attempt to resolve ambiguities that arise from either data set alone. The input for LEFSA is the molecular weight of the peptide, the CID m/z and abundance values, and a listing of amino acids found in each cycle of Edman degradation. From the molecular weight and the Edman data, a limited set of possible sequences is generated for each peptide. The sequences in this list are then scored according to how well they account for the CID fragmentations. Shown in Figure 1 are the amino acids found in each cycle of Edman degradation for a HPLC fraction of a tryptic digest of cytochrome c from yeast. This information plus the CID spectra of three of the peptides present in the mixture gave the results listed at the bottom of Figure 1. The correct sequences were ranked first for each peptide, but most noticeable was the wide range of scores for the sequences listed, none of which had identical scores. In addition, leucine and isoleucine could be differentiated, and there were no unsequenced pairs of amino acids. This is in contrast to what was found when only CID data was considered.

Table I: Results From Twenty Peptides using LUTEFISK

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Time</th>
<th>Rank</th>
<th>Fragment #</th>
<th>Charge</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLPR</td>
<td>1s</td>
<td>1(1)</td>
<td>17</td>
<td>2</td>
<td>0.907</td>
</tr>
<tr>
<td>YFKI</td>
<td>1s</td>
<td>1(1)</td>
<td>11</td>
<td>1</td>
<td>0.779</td>
</tr>
<tr>
<td>LMNT</td>
<td>1s</td>
<td>1(1)</td>
<td>10</td>
<td>1</td>
<td>0.900</td>
</tr>
<tr>
<td>YLDShq</td>
<td>1s</td>
<td>1(1)</td>
<td>18</td>
<td>1</td>
<td>0.814</td>
</tr>
<tr>
<td>GSVIQK</td>
<td>3s</td>
<td>1(1)</td>
<td>28</td>
<td>2</td>
<td>0.987</td>
</tr>
<tr>
<td>EVVDPTK</td>
<td>3s</td>
<td>1(1)</td>
<td>22</td>
<td>2</td>
<td>0.957</td>
</tr>
<tr>
<td>AQDFVQW</td>
<td>2s</td>
<td>3(11)</td>
<td>13</td>
<td>1</td>
<td>0.868</td>
</tr>
<tr>
<td>GIGALKV</td>
<td>7s</td>
<td>1(2)</td>
<td>24</td>
<td>2</td>
<td>0.989</td>
</tr>
<tr>
<td>ITGGLPAl</td>
<td>4s</td>
<td>1(1)</td>
<td>32</td>
<td>1</td>
<td>0.972</td>
</tr>
<tr>
<td>DRVYVHFP</td>
<td>30s</td>
<td>5(8)</td>
<td>29</td>
<td>2</td>
<td>0.958</td>
</tr>
<tr>
<td>DRVYVHFP</td>
<td>30s</td>
<td>1(2)</td>
<td>34</td>
<td>3</td>
<td>0.972</td>
</tr>
<tr>
<td>PPGFSPF</td>
<td>29s</td>
<td>1(1)</td>
<td>46</td>
<td>2</td>
<td>0.832</td>
</tr>
<tr>
<td>NPGFSPFR</td>
<td>29s</td>
<td>2(2)</td>
<td>34</td>
<td>2</td>
<td>0.877</td>
</tr>
<tr>
<td>RPPGFSPFR</td>
<td>37s</td>
<td>1(1)</td>
<td>49</td>
<td>2</td>
<td>0.851</td>
</tr>
<tr>
<td>DRVYLVHPFL</td>
<td>40a</td>
<td>------</td>
<td>35</td>
<td>2</td>
<td>------</td>
</tr>
<tr>
<td>HTLQISQV</td>
<td>27s</td>
<td>1(1)</td>
<td>45</td>
<td>2</td>
<td>0.873</td>
</tr>
<tr>
<td>QDQVQVLTFTK</td>
<td>27s</td>
<td>1(1)</td>
<td>34</td>
<td>2</td>
<td>0.915</td>
</tr>
<tr>
<td>HSQGTQFTSDYSK</td>
<td>11s</td>
<td>1(4)</td>
<td>32</td>
<td>2</td>
<td>0.922</td>
</tr>
<tr>
<td>DRVYLVHPFL</td>
<td>2m</td>
<td>1(2)</td>
<td>63</td>
<td>3</td>
<td>0.943</td>
</tr>
<tr>
<td>TPGQVSIPGFR</td>
<td>23s</td>
<td>1(2)</td>
<td>24</td>
<td>2</td>
<td>0.973</td>
</tr>
<tr>
<td>NDDLM(0)TSSVRASM(0)</td>
<td>4s</td>
<td>2(2)</td>
<td>32</td>
<td>2</td>
<td>0.891</td>
</tr>
</tbody>
</table>

Figure 1: Sequencing with LEFSA
Gas Phase Edman Data for Yeast Cytochrome C Tryptic Digest HPLC Fraction 4:
Cycle no. 1 2 3 4 5 6 7 8 9 10 11 12 13
D D N T I L K L K R R R
G R L Q L H T V G
E I A V E

Peptide 1: MW 1165.6 Peptide 2: MW 978.5 Peptide 3: MW 1249.6
0.971 VGPNLHGLGFR 0.807 NDLITYLKL 0.944 DRNDLITYLKL
0.923 VGPNLHGFYY 0.771 NDLITYLKL 0.871 DRNDLITYLKL
0.848 VGPNLTYKFR 0.740 NDLITYLKL 0.822 DRLQTYLKL
0.822 VGPNLGYFGR 0.654 GGLDYTLKL 0.821 DRQTYLKL
0.814 VGLNAYGIFGR 0.654 GGNNLYTLKL 0.805 DRNDLITYLKL
0.814 VGLNAYGFGR 0.650 NDLQTYLTI 0.786 NNQTITYLKL
0.798 VGPQAIKGLKR 0.644 NDLQTYLTI 0.780 DRNDLITYLKL
0.794 VGPNTYLFKF 0.635 NDLQTYLTI 0.778 VRNDLITYLKL
0.793 VGPQIOGLKLKR 0.629 NDLQTYLTI 0.778 VRQTYLKL
0.792 VGPNAALKLKR 0.601 NDLDAATIF 0.774 ERNNTITYLKL
0.785 VGNIAAYGFY 0.597 NDLNTAGKF 0.774 DRNNTITYLKL
0.785 VGPQAIYTFGR 0.589 GGLDLETKFL 0.767 ERLNTITYLKL
0.783 VGPNAAYGLGR 0.589 GGNIBLKLFR 0.751 ERFNTYLKL
0.783 VGLNANYGYF 0.546 VDQTYLKL 0.751 NRTLITLKL
0.781 VGPAYGKFGFR 0.528 GGLDDATLGY 0.747 DDNQLELYLKL
0.779 VGPIILTKLGR 0.528 GGNNTAGILY 0.745 ERNDAEYLYLKL

1234
A very important characteristic of any analytical instrument is the range of analyte concentration over which the output signal shows a response that is linear with concentration. Almost from the relatively recent beginning of Electrospray Mass Spectrometry (ESMS) it has been realized that the dependence of ion current upon analyte concentration in the sample solution shows a rather abrupt change in slope at some critical value of that concentration. The reasons for this behaviour have never been clearly identified and are addressed here.

Figure 1 illustrates the phenomenon for the cyclic peptide Gramicidin S in a 1:1 water solvent. The analyte ion current is linear in concentration from 1 pg/μL to 10 ng/μL, four orders of magnitude. Then it shows a fairly abrupt decrease in slope to nearly zero, indicating saturation of some sort. This behavior is typical of all the species we have examined. The main differences from one species to another are (1) the value of the concentration at which the change in slope occurs and (2) the trend of the slope after the changes. For some species it actually turns negative showing a decrease in ion current with increasing analyte concentration. When the mass spectrum comprises number of peaks for a particular species, e.g. because of multiple charging, one should sum over those peaks to determine the total contribution to ion current for that species. In such cases we do not know how much multiplier response may be affected by the number of charges per ion. Consequently, the true contribution of multiply charged ions of each peak to the total ion current for that species is at best an approximation.

This change-in-slope has sometimes been attributed to a charge limitation. When analyte molecules in a droplet begin to outnumber charges on the droplet, further increases in concentration cannot increase the ion production. But this explanation doesn't stand up to close inspection. For example, plots like those of Fig. 1 for tetramethyl, tetrabutyl and tetraheptyl quarternary ammonium halides show that in each case the slope change occurs at the same weight per cent concentration of analyte corresponding to seven-fold change in molarity. Since the ions in each case are singly charged, smallest of these species should show a slope change at one seventh of the concentration of the largest, if charge limitation is the cause. More evidence that charge limitation cannot be the cause is shown in Fig. 2 which is the equivalent of Fig. 1 for Cyclosporin A (M = 1202), a cyclic peptide of nearly the same size as Gramicidin S (M = 1140). Although there are not enough data points to define the slope change point with any precision, it is clear that it occurs at a lower molar concentration for Cyclosporin than for Gramicidin. Moreover, mass analysis shows that most of the former ions are singly charged, whereas most of the latter are doubly charged. Shortage of charge cannot account for this difference.

An even more dramatic challenge to the charge limitation explanation is shown in Fig. 3. The solid curve is for tetrabutyl ammonium bromide in 1:1 water. The dashed curve is the same except that 2% acetic acid has been added, resulting in a shift of the slope change concentration of nearly two orders of magnitude! It turns out that addition of NaOH shifts the slope change concentration in the opposite direction, i.e. to a lower value than for the solid curve. To be remembered is that the solubility of ammonium salts generally increases with decreasing pH of the solvent. Also to be noted is that Cyclosporin is more insoluble in water and alcohol than in Gramicidin. We conclude that the slope change point in graphs like those of Figs. 1, 2 and 3 is most likely determined by the solubility of
the analyte. When the droplet liquid is saturated with analyte, addition of more, or removal of solvent, cannot increase its concentration.

Fig. 1

Fig. 2

Fig. 3
Electrospray (ES) ionization is rapidly developing as a method to produce gas-phase ions from analyte species in solution for subsequent analysis by mass spectrometry (MS). The ES-MS combination has proven useful in the analysis of involatile, polar, and thermally labile compounds, especially high molecular weight biopolymers, and also serves to interface the mass spectrometer with a variety of liquid phase separation methods. While the detailed mechanism for ion evaporation or ion desorption is currently at issue, it has become clear that best ES-MS results, both in terms of sensitivity and detection limits, are achieved for compounds that are ions in solution. Species that are ionic in solution and have been analyzed by ES-MS include, for example, metal salts and organic salts. Compounds with functionalities that can be ionized via solution phase acid/base chemistry, such as carboxylic acids and amines, are also amenable to ES-MS. The latter category of compounds includes peptides and proteins which contain basic amino acid residues and oligonucleotides which contain acidic phosphate groups and are usually detected as the (M+H)+ and (M+nNa)+ species, respectively. Some polar molecules are also ionized efficiently by ES via attachment of ions other than a proton (e.g., Na+ or CH3COO−) that are present in the analyte solution.

Current and potential methods for preforming ions in solution for ES-MS can be divided into three broad categories, viz., chemical, electrochemical and photochemical methods. To date only the acid/base solution chemistry of an analyte has been exploited to preform ions. As a result, the applicability of ES ionization is currently limited to compounds that are ionic in solution or that can be ionized in solution (or in the desolvating droplets) by acid/base chemistry or by adduct formation. Several important classes of compounds, such as the polycyclic aromatic hydrocarbons (PAH's), cannot, at present, be analyzed using this technique. Additionally, the ions formed in solution via acid/base chemistry or adduct formation are limited in positive ion mode to cationized species and in negative ion mode to the deprotonated molecule or an adduct ion. Radical cations, M•+, and radical anions, M•−, which directly provide the molecular weight and might be of use in other mass spectrometric analyses, such as MS/MS, are typically not formed by ES ionization. In this paper, charge-transfer (CT) complexation and electrochemical reactions are demonstrated as means with which to form radical cations or anions in solution, both for compounds that display acid/base behavior and also for compounds that are otherwise not amenable to analysis by ES-MS. The data described in this paper were acquired on an ES/ion trap mass spectrometer combination that has been described in detail (1).

CT complexes are formed by electron transfer between an electron donor (D) and electron acceptor (A) as shown in equation 1

\[ D + A \rightarrow (D^+\cdot A^-) \rightarrow (D^+)^{\text{base}} + (A^-)^{\text{base}} \]  

Thus, this method can be used to form both radical cations and anions from species that undergo these reactions. Electron donors and acceptors are usually compounds of low ionization energy (IE) and compounds of high electron affinity (EA), relative to one another, respectively. Typical electron donors include PAH's and other aromatic species that contain electron-donating groups such as -OH, -OCH2, and -CH3. Common electron acceptors usually contain several electron withdrawing groups, such as -NO2, -CN, or halides. For example, the quinones chloranil (tetrahydro-p-benzoquinone) and 2,3-dicyano-1,4-benzoquinone (DDQ) are good acceptors. Figure 1 shows the positive and negative ion ES mass spectra obtained from a sample containing a mixture of the donor N,N',N'-tetramethyl-1,4-phénylenediamine (TMPD) and the acceptor DDQ. Without the addition of DDQ, the spectrum of TMPD shows the protonated molecule as the major ion. When DDQ is added, the radical cation is the ion observed. In negative ion mode, the molecular anionic species observed corresponds in mass to (DDQ+1)−. This observation suggests that the original molecular anion formed from DDQ by CT complexation with TMPD undergoes reactions in solution. The positive ion ES spectra of 2,3-benzanthracene in Figure 2 demonstrate that CT complexation can be used to ionize PAH type compounds which normally give no signal in ES-MS.

Electrochemistry can also be used to form ions in solution for ES. The setup used in these experiments, shown in Figure 3, consisted of an electrochemical LC detector in which both electrodes of the cell were floated at the high voltage necessary to promote ES. With this setup, no high voltage ES needle is required since the solution is charged in the cell. Figure 4 shows the electrochemically assisted ES spectra acquired for the perylene. When the voltage difference between the two electrodes in the cell is zero (Cell OFF) no signal is observed. However, when the two electrodes are offset by 1.5 V (Cell ON), the radical cation of perylene, formed by the electrochemical oxidation of the analyte within the cell, is observed.

In summary, both CT and electrochemistry are viable methods for preforming ions for ES-MS. The sensitivity and detection limits for these methods are comparable to those limits obtained for compounds analyzed using conventional ES techniques. In addition, both of these methods are potentially useful in on-line applications. Although both techniques will work for only a limited set of compounds, they do provide compound specific ionization, can be used to ionize nonpolar, neutral compounds, and also provide a means to form radical cations/anions via ES.
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Figure 1

ES Mass Spectra of a TMPD/DDO Mixture

Positive Ion Mode

CHCl₃/methanol (50/50 v/v)
46 pmol/µL TMPD
55 pmol/µL DDQ
0.82 pmol of each consumed

Negative Ion Mode

CHCl₃/methanol (50/50 v/v)
46 pmol/µL 2,3-benzanthracene
56 pmol/µL DDQ
0.62 pmol of both consumed

Figure 2

Positive Ion ES Mass Spectra of 2,3-benzanthracene

2,3-benzanthracene only
CHCl₃/methanol (50/50 v/v)
46 pmol/µL 2,3-benzanthracene
0.62 pmol consumed

Figure 3

Electrochemically Assisted Electrospray Setup

EC Cell OFF

EC Cell ON 1.5 V

CHCl₃/0.1% TFA
79 pmol/µL Perylene
21 pmol sampled

Figure 4

EC Assisted ES Mass Spectra of Perylene
Is the Electrospray Mass Spectrum of Proteins Related to their Aqueous Solution Chemistry?

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The fundamental shape of the ion abundance profile observed in electrospray of proteins is controlled by the concentration of pre-formed ions in aqueous solution. An aqueous solution equilibrium model based on the pKa's of acidic and basic sites on proteins has been developed to account for the shape of the ion abundance profile in the electrospray mass spectrum. Monotonic functions such as mass spectrometric transmission efficiency may alter the centroid of the profile, but the basic factor that controls the ion abundance pattern is aqueous acid/base solution chemistry. Electrospray measurements were performed using a TAGA 6000E (SCIEX Inc., Thornhill, Ont. Canada) triple quadrupole mass spectrometer. To ensure reliable measurement of relative ion abundance data, the mass resolution was decreased from unit mass resolution to the extent that two or more measurements were taken near the apex of every peak. Samples were continuously infused at a rate of 20nL/mln through a 100μm ID stainless steel capillary held at 3.5kV. Stable operation of electrospray was achieved with the needle approximately 1.5 cm from the instrument orifice, and with a measured stable current of between 20 and 50 nA. Cytochrome c (horse heart, C-2506 type III) and myoglobin (horse skeletal muscle, M-0630) were obtained from Sigma Chemical Co. (St. Louis MO, USA). Cytochrome c samples (20 μg/ml) were prepared to cover various pH ranges; from pH 2.5 to 3.6 with HCl, from pH 2.8 to 3.5 with formic acid, and from pH 3.5 to 3.9 with acetic acid. Each of these cytochrome c solutions was diluted 1:1 with methanol. Similarly, three series of myoglobin solutions (20 μg/ml) were prepared; from pH 2.9 to 3.7 with HCl, from pH 2.7 to 3.3 with formic acid and from 3.2 to 3.6 with acetic acid. In turn, each solution was diluted 1:1 with methanol.

In aqueous solution the protonation of carboxylic acid groups controls the net charge on proteins in solutions below pH 5 [1]. The pKa's of the carboxylic acid groups in cytochrome c can be estimated. For example, assume that one of the carboxylic groups has a pKa of 3.9 (i.e. the pKa of the carboxylic group of aspartic acid) at the point wherein the mass to charge ratio of the cytochrome c ion has reached 1550. From this point, the protonation of each subsequent carboxylic acid will increase the net charge on the protein, increase the electrostatic repulsion on approaching protons, and therefore lower the pKa of each subsequent carboxylic group (e.g. cytochrome c, low ionic strength, each pKa is 0.086 [1,2] unit lower). On the basis of this simple array of pKa values, the abundance of the ions in aqueous solution is calculated. More refined models have been described [1]. A comparison of calculated and experimental abundance profiles are shown in Fig.1 and 2. The ion abundance pattern of myoglobin (Fig.2) is more complex than that of cytochrome c. Under some experimental conditions it appears to be a sum of two functions (i.e. two distinct tertiary structures which have unique sets of pKa values). Cytochrome c will also exhibit this dual function distribution in solutions containing little methanol and/or acid, but not under the experimental conditions used to collect the data in Figure 1.

The ion transmission efficiency of a quadrupole mass spectrometer is a function of mass. Previous estimates [3] of the transmission roll-off of a SCIEX TAGA instrument of this type have been as high as an order of magnitude per 200 m/z units. Fig.3 illustrates the ion abundance *envelope* for a set of experimental data for cytochrome c and a calculated profile using a solution pH 3.3. This profile was then modified by the transmission roll-off. With an estimated roll-off function of about a factor of 5 per 200 m/z units, the resulting profile matched the experimental data. The fundamental origin of the ion abundance profile observed in electrospray is controlled by the concentration of pre-formed ions in aqueous solution. Other monotonic exponential increasing, or decreasing, functions can shift this original pattern, nevertheless the abundance pattern observed in the mass spectrum retains the shape imposed by aqueous solution chemistry.

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Cytochrome c

![Ion abundance profiles of multiply charged cytochrome c: *, experimental results; +, profiles calculated from the aqueous solution equilibrium model.](image)

Myoglobin

![Ion abundance profiles of multiply charged myoglobin: *, experimental results; + and Δ profiles calculated from the aqueous solution equilibrium model; o sum of solution equilibrium profiles.](image)

Cytochrome c

![Effect of ion transmission efficiency on the abundance profile of cytochrome c: * experimental results; + calculated profile at pH 3.33; o calculated profile at pH 3.33 plus a transmission roll-off of 5.2/200 m/z units.](image)

1240
Obtaining Structural Information from Electrospray ionization-Mass Spectrometry at the Large Molecule Limit

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A number of interesting challenges must be met if one is to obtain structural information from very large molecules. Molecular ions in predominantly low charge states, such as those produced by matrix-assisted laser desorption, are difficult to collisionally activate (unless collision energies are greatly increased) and one must ultimately rely on photoexcitation. The pulsed nature of the laser desorption and photodissociation processes makes this combination well suited for TOF methods, but appears to place experimental constraints upon resolution and m/z range. Additional pragmatic constraints arise, however, due to the inherent isotopic distribution and the actual molecular weight (MW) distribution of high molecular weight substances (due to small sequence variations). This and other sources of "peak splitting" lead to spectral congestion as revealed in the apparent "peak widths" of most mass spectra for biomolecules of MW > 30,000. In contrast, electrospray ionization (ESI) can produce highly charged molecular ions that can be efficiently activated by collisions regardless of MW if their charge state is sufficiently high. We have already obtained substantial sequence-related information for albumin molecular ions of ~66,000 MW. We propose a new approach, single molecule analysis by mass spectrometry (SMAMS), with the aim of allowing molecules in the megadalton range to be analyzed by their sequential dissociative processes. Ion cyclotron resonance (ICR) methods may provide an especially suitable approach for such studies due to the potential for high sensitivity and resolution for ions of high charge, the potential for high mass measurement accuracy and the ability to "re-use" ions. Our current plans for moving in this direction are summarized.

In ICR sensitivity is proportional to the number of charges in the cell. the practical sensitivity limit for analytical ICR instruments is presently ~10 charges. It matters not whether one has ten singly charged ions in the cell or a single ion with ten charges. Thus single ion detection should be readily achievable for ions containing more than ten charges. Electrospray readily generates ions containing tens or even hundreds of elementary charges. Therefore, single highly charged ions should be readily detected in ICR using detection technology presently available. Image current detection in ICR is non-destructive, so ions may be re-used for sequential reactions as has already been demonstrated by a number of investigators. If a single ion is followed through a set of sequential reactions (i.e., fragmentations) special advantages are obtained. These primarily have to do with a tremendous simplification of the spectra. There is no longer an ensemble of ions of slightly varying m/z (due to isotope distributions, etc.) but rather a single ion with single values for mass and charge. In SMAMS the requirements for resolution may be substantially reduced compared to conventional techniques. With only a single ion present in the trap, there are no interfering "isotope peaks" and the m/z of the ion can be confidentially assigned by knowing the centroid of the peak alone. Furthermore, when this ion fragments it must "choose" a single one of many possible fragmentation spectra. Thus, the dissociation spectrum is also very simple and could perhaps be interpreted in real time. This allows the possibility of a "real-time adaptive" strategy in which the results of the last dissociation event help guide conditions (such as re-excitation frequency) for the rest of the next sequential step. Perhaps the most significant (and least obvious) advantage of SMAMS is that it may relax the requirement for m/z accuracy. For example, suppose one can select conditions favoring the sequential loss of residues from the 3' end of a highly charged DNA ion, and one continues to observe the m/z of the larger fragment as the system undergoes sequential dissociations. In order to sequence the DNA one is only required to differentiate between the loss of adenine and thymine, a difference of nine daltons. Even allowing...
for as many as four deuterium substitutions on the leaving group, one would need only a five dalton specification for mass accuracy. A side benefit is that errors in sequence assignment are localized to single residues and do not propagate through the system. The feasibility of SMAMS depends in part upon the ability to degrade the system under study in a series of highly predictable steps. Some general strategies for achieving this are currently being explored, but more research is clearly needed into selective gas phase degradation processes.

Pacific Northwest Laboratory is operated for the U.S. Department of Energy by Battelle Memorial Institute under Contract DE-AC06-76RLO 1830. This research has been supported internally by the Molecular Sciences Research Center and the Environmental and Molecular Sciences Laboratory at Pacific Northwest Laboratory.
Production of multiply charged molecular ions by electrospray ionization (ESI) has allowed mass spectrometry (MS) to obtain structural information for large proteins by collisionally activated dissociation (CAD) processes. CAD of intact molecular ions with subsequent MS\textsuperscript{n} (\(n \geq 2\)) analysis of the resulting product ion is potentially a more rapid and sensitive method for amino acid sequence analysis. On a triple quadrupole mass spectrometer, CAD of ESI-generated ions can be induced in the atmospheric pressure/vacuum interface or, more traditionally, in the collision quadrupole cell (Q2). Combining these two processes, dissociation of molecular ions in the interface region (with an elevated voltage difference between the nozzle and skimmer) and further dissociation of the product ions in Q2 (an MS/MS/MS experiment), provides an effective means for examining molecular regions unprobed by MS/MS and further confirmation of mass spectral assignments. In principle, nearly complete sequence information for relatively large species can be provided with such MS\textsuperscript{3} experiments, although the limitations of current instrumentation make interpretation of CAD mass spectra of multiply charged ions for true "unknowns" problematic. Tandem mass spectra of multiply charged ions are often dominated by several series of product ions with various charge states. For example, MS/MS of the (M+18H)\textsuperscript{+} molecular ion of human growth hormone (Mr 22 kD) yields b\textsubscript{n} sequence ions with 3+, 4+, and 5+ charge states. Deconvolution methods used for Mr measurements from ESI mass spectra can be applied to such product ion mass spectra.

Comparing data from samples with minor structural modifications can simplify the spectral interpretation procedure. Transferrin glycoproteins (Mr 78 kD) from various species have been examined by CAD, yielding significant sequence information (typically from the NH\textsubscript{2} terminus). Serum albumin proteins (Mr 66kD) from 10 different species (bovine, human, rat, horse, sheep, dog, goat, porcine, and guinea pig) have been examined by ESI-MS, MS/MS, and MS/MS/MS. The primary dissociation product ions assigned have low charge state (2+ to 5+) and are attributed to "b\textsubscript{n}" mode species from cleavage of the -CO-N- peptide backbone bonds. Particularly intense dissociation products originate from regions near residues \(n = 20-25\) from the NH\textsubscript{2}-terminus for parent ions of moderate charge (\(-50+\)). Collisionally activated dissociation mass spectra from porcine serum albumin, in contrast to the other albumins, also give prominent singly charged "y\textsubscript{n}" fragments formed from cleavages near the COOH-terminus. Tandem mass spectrometry of the multiply charged molecular ions, and of fragment species produced by dissociation in the interface produced similar "b\textsubscript{n}" species and served to confirm spectral assignments.

Protein structural factors, such as the presence of proline residues, can have dramatic effects on the collisional dissociation spectrum. Proline is unique among the common amino acids in that the side chain imposers rigid constraints on the N-C rotation. It has been previously observed in CAD mass spectra of (M+H)\textsuperscript{+} from small peptides that cleavage is sometimes favored on the NH\textsubscript{2}-terminal side of a proline bond. Dissociation of multiply charged ions of peptides and proteins under low energy collision conditions often show this enhanced fragmentation adjacent to a proline residue. For example, CAD of carbonic anhydrase (259 residues, Mr 29 kD) yields multiply charged products primarily in the vicinity of four proline residues near the COOH-terminus (residues 193, 199, 213, and 225). Bovine ubiquitin (Mr 8564) dissociates primarily between Glu(18)-Pro(19) to yield highly charged y\textsubscript{n} ions. The sequence for yeast ubiquitin involves a substitution of Ser for Pro(19). Dissociation products are more evenly distributed in the vicinity of residues 14-21.
Interpretation of such collisional dissociation spectra for "unknowns" is currently difficult due to the complexity of the spectra and the uncertainty of assignments for product ion charge states. With higher resolution methods (e.g., Fourier transform MS), charge state assignments from resolved isotopic peaks is possible. Such methods would largely circumvent the limitations of the present triple quadrupole methods, suggesting the potential for extension to even greater molecular weights.

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MOLECULAR WEIGHT DETERMINATION OF DEUTERATED RIBOSOMAL PROTEINS BY ELECTROSPRAY IONIZATION

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Electrospray ionization mass spectrometry (ESI MS) has become an invaluable research tool for its ability to determine the molecular weight of picomole concentrations of large and labile biomolecules. This paper presents how ESI MS can be used in conjunction with neutron scattering experiments for the structural determination of ribosomes.

Precise determination of percent deuteration of nonexchangeable protons in the specific ribosomal protein is necessary prior to costly neutron scattering experiments. Typically, this is determined using expensive, and time consuming contrast variation method which can consume up to micromole amounts of sample as opposed to the picomole concentrations typically used in ESI MS.

In neutron scattering experiments, after individual proteins are purified from the ribosome, all but few of the ribosomal proteins are deuterated, and added together with the RNA to reconstruct the ribosome. Since the neutron scattering intensity from the deuterium containing ribosomal proteins will vary from that of naturally occurring ribosomal proteins, the individual proteins can be selectively mapped on the ribosome by observing the neutron scattering angles and the intensity. One of the most widely studied ribosomes to date is the E. coli ribosome which consists of two subunits that has a total of 3 rRNA and 52 proteins. Although the molecular weight and structural sequence of each individual protein is studied very well, little is known about their functions in the ribosome. By spatially determining the subunits of the ribosome and in turn their complicated assembly of RNAs and proteins, neutron scattering can help to understand the functions of the individual components of the ribosome.

Fully deuterated, protonated and partially deuterated S4 ribosomal proteins from a 30S E. coli subunit were purified and diluted in 1:1 Methanol:Water solution in 0.1\mu g/\mu l concentration. The samples were introduced at 1\mu l/min flow rates (approx 4 pmoles/min) into an Analytica electrospray ionization source interfaced to an HP 5988 quadruple mass spectrometer (Figure 1). Figure 2 shows ESI mass spectrum of each of the three proteins taken in 2 minute scans (total of 8 to 10 pmoles of sample).

The S4 ribosomal protein has a theoretical monoisotopic mass of 23123.5 amu with 1279 nonexchangeable hydrogen sites in solution. In order to find the percent deuteration of the S4
protein, the molecular weight of all three protonated, deuterated, and partially deuterated proteins are compared to each other under the same experimental conditions. Table 1 summarizes the experimental results and compares these to the calculated results. From the synthesis, the partially deuterated ribosomal S4 protein was expected to be around 50%; indeed, the experimental results agree with the predicted percentage showing confidence not only in the purity of the protein but also confidence in using this number as an important parameter for the neutron scattering experiment.

The ESI mass spectra of Figure 2 were analyzed with the recently developed deconvolution computer program. The new algorithm greatly simplifies the deconvolution routine by, first, finding the coherence between the multiply charged peaks, and then, assigning the molecular weight of the macro molecule. The calculations do not assume the conventional proton adduct ion in finding the macro mass. All possible adduct ion masses are considered, and their intensity contributions to the respective macro masses are plotted in a three dimensional deconvolution spectrum.

<table>
<thead>
<tr>
<th></th>
<th>S4 P</th>
<th>S4 D/P</th>
<th>S4 D</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXPERIMENT</td>
<td>23123.5</td>
<td>(23766.9)</td>
<td>(24410.2)</td>
</tr>
<tr>
<td>MW</td>
<td>23410</td>
<td>24044</td>
<td>24637</td>
</tr>
<tr>
<td>EXP. - CALC.</td>
<td>286 (+1.2%)</td>
<td>277 (+1.2%)</td>
<td>227 (+0.9%)</td>
</tr>
<tr>
<td>S4D-S4P</td>
<td>1227</td>
<td>1286.7</td>
<td></td>
</tr>
<tr>
<td>S4D/P - S4P</td>
<td>634</td>
<td>643.4</td>
<td></td>
</tr>
<tr>
<td>%D</td>
<td>52%</td>
<td>50%</td>
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</table>

**TABLE I**
CONFORMATIONAL CHANGES IN PROTEINS PROBED BY HYDROGEN EXCHANGE ELECTROSPRAY IONIZATION MASS SPECTROMETRY

Viswanatham Katta and Brian T. Chait
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When peptides and proteins are dissolved in solvents like D_2O, labile hydrogens exchange with deuteriums. The exchange rates differ widely between different labile hydrogens and are a strong function of pH (slowest at pH about 3). In small peptides, in the absence of high order structure, all the labile hydrogens are accessible to the solvents and, even for the slowly exchanging amide backbone hydrogens, the exchange is complete in minutes. The same is expected of proteins in unfolded conformations (denatured states). For proteins in tightly folded conformations (native states), some of the labile hydrogens are inaccessible to the solvent because they are buried in the hydrophobic core or involved in salt bridges and hydrogen bonds, and are not readily available for exchange. The rates of exchange of these hydrogens can differ by orders of magnitude depending on the conformation of protein in solution. Thus, native and denatured conformations exhibit different exchange kinetics. After a short interval of exchange, the folded and unfolded proteins will differ in the degree of deuterium incorporation that has occurred and end up with different molecular masses.

The availability and the effective pKs of the acidic and basic side chains are determined by the precise conformation that the protein assumes under the conditions of study. As a result tightly folded native states will have lower net positive charge than the denatured states in solution. The multiply charged ions observed in the positive electrospray ionization (ESI) spectra reflect, to some extent, the degree of protonation in solution. Thus, a combination of hydrogen exchange with electrospray ionization is a natural choice for probing conformational changes because different forms of proteins will exhibit different masses and charge distributions in their mass spectra.

Bovine ubiquitin, in its native form is a small, tightly folded protein (76 residues, molecular mass 8565, no disulfide bonds, 13 basic sites, 144 labile hydrogens) and is very resistant to denaturation. Figure 1 compares the ESI mass spectra of ubiquitin obtained (a) from a solution in which the protein is known to be in a tightly folded conformation (1% CH_3COOD in D_2O) and (b) from a solution in which the protein is known to be unfolded (1:1 CH_3OD:1% CH_3COOD in D_2O). The spectra were obtained about 20 minutes after dissolving the protein in the above solutions (temperature 23 °C). The spectrum obtained from the native protein shows a charge distribution centered at 8+ (range 9+ to 6+) and the measured mass indicated an exchange of only 89 hydrogens (i.e. 62% in 20 minutes). On the other hand the spectrum obtained from denatured ubiquitin showed a distribution centered at 10+ (range 13+ to 6+) and a mass indicative of an exchange of 131 hydrogens (i.e. 91% in 23 minutes). The exchanges were followed as a function of time. In the case of the folded protein the exchange slowed considerably after 90 minutes (103 exchanges) and only 130 exchanges even after a week. In the case of the unfolded protein, almost complete exchange (138 exchanges) was observed within 60 minutes.

The method was applied for probing other protein conformations and the results are tabulated in Table I. Chicken egg lysozyme (molecular mass 14305, four disulfide bonds, 19 basic sites, 255 labile hydrogens) showed a charge distribution centered at 10+ (range 11+ to 8+) and an exchange of only 62% in 30 minutes, suggesting that the protein is in native state in 1% acetic acid. The spectrum obtained from lysozyme in which the -S-S- bonds were reduced, showed a distribution centered at 15+ (range 19+ to 9+) and indicated an exchange of 96% of the 263 labile hydrogens, suggesting that the reduced protein is denatured in this solvent (1% acetic acid). Other proteins listed in the table could be analyzed only in the denatured states (greater than 94% exchange in 30 minutes). Ions produced from the native states could not be observed, presumably because the m/z values of the low charge states of these ions were beyond the range of our instrument (m/z 2000).

The results demonstrate that hydrogen exchange electrospray ionization mass spectrometry provides an effective new method for probing protein conformations in solution.

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**Bovine ubiquitin**

1% CH$_3$COOD in D$_2$O

Exchanges 00 (144) 62% in 20 min.

8653.1

7+ 8654.3

6+

Figure 1

CH$_3$OD : 1% CH$_3$COOD (1 : 1)

Exchanges 131 (144) 91% in 23 min.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Solvent</th>
<th>Number of possible exchanges</th>
<th>Percent exchanged in 30 min.</th>
<th>State</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovin. ubiquitin</td>
<td>1% CH$_3$COOD in D$_2$O</td>
<td>144</td>
<td>65%</td>
<td>Native</td>
</tr>
<tr>
<td>Bovin. ubiquitin</td>
<td>CH$_3$OD : 1% CH$_3$COOD in D$_2$O</td>
<td>144</td>
<td>84%</td>
<td>Denatured</td>
</tr>
<tr>
<td>C.S. lysozyme (native)</td>
<td>1% CH$_3$COOD in D$_2$O</td>
<td>258</td>
<td>62%</td>
<td>Native</td>
</tr>
<tr>
<td>C.S. lysozyme (reduced)</td>
<td>1% CH$_3$COOD in D$_2$O</td>
<td>263</td>
<td>90%</td>
<td>Denatured</td>
</tr>
<tr>
<td>Bovin. carbonic anhydrase</td>
<td>2% CH$_3$COOD in D$_2$O</td>
<td>450</td>
<td>99%</td>
<td>Denatured</td>
</tr>
<tr>
<td>Equine myoglobin</td>
<td>1% CH$_3$COOD in D$_2$O</td>
<td>252</td>
<td>95%</td>
<td>Denatured</td>
</tr>
<tr>
<td>Bovin. hemoglobin</td>
<td>CH$_3$OD : 2% CH$_3$COOD in D$_2$O</td>
<td>228(p)</td>
<td>87%</td>
<td>Denatured</td>
</tr>
</tbody>
</table>

Table 1
Simultaneous Determination SQ 28,555 and SQ 27,519 in Serum by High Performance Liquid Chromatography Combined with Ionspray Mass Spectrometry.

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New Brunswick, New Jersey 08903

SQ 28,555 is a second generation angiotensin converting enzyme (ACE) inhibitor and is currently under clinical investigation as a treatment for hypertension. It is a pro-drug which is hydrolyzed in vivo to its active form SQ 27,519. The purpose of this study was to develop a sensitive and specific assay for these two compounds based on ionspray LC/MS.

Initially, negative ion detection was attempted. Since both compounds contain a free carboxylic acid, they should exist as anions in solution at neutral pH. However, while negative ion spectra showed good sensitivity for SQ 27,519, poor results were obtained for SQ 28,555. The main difference between these two compounds is that the phosphinic acid is blocked in the pro-drug and free in the active compound. As a result, the use of (M+NH4)+ for each analyte was found to be a better overall choice. Such a cluster could be easily and reproducibly generated by maintaining a relatively low orifice potential of +45V. Spectra collected under these conditions showed predominant (M+NH4)+ species not only for the analytes, but also for the structural analogues that were used as internal standards.

Serum samples were prepared by liquid-liquid extraction using purified ethyl acetate. The dried extracts were dissolved in loading buffer and separations were carried out by gradient chromatography on a 2mm ID Deltabond C8 column. The column was operated at 60° C, which improved the chromatographic peak shape of SQ 27,519 by increasing the rate of cis-trans interconversion of the proline portion of the molecule. Column temperature had no effect on the chromatography of SQ 26,555 under these conditions. The flow rate was 300 μL/min with a 1 to 6 split introduced to the ionspray interface. All compounds eluted within six minutes and the total analysis time was ten minutes.

The serum extracts proved to be more contaminated than anticipated making low level quantification difficult. Daughter ion spectra were collected for the compounds and multiple reaction monitoring (MRM) experiments were analyzed. The daughter ions monitored for both compounds were (M+H)+ of SQ 27,519, representing the loss of NH3 from both compounds in addition to the loss of the blocking group from SQ 28,555. Some interference was still present in the SQ 27,519 channel, but the SQ 28,555 trace was clean. If a more specific reaction could have been generated for SQ 27,519, perhaps the interference would have been eliminated, but the molecule proved to quite stable. No hydrolysis of SQ 28,555 to SQ 27,519 was evident due to extraction or analysis conditions.

The system was linear from 5 ng/mL to 1000 ng/mL and validation data demonstrated good accuracy and reproducibility.
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SIM m/z 453 + 581

5 ng/mL

MRM m/z 453/436 + 581/436

5 ng/mL

Accuracy/Precision Results

<table>
<thead>
<tr>
<th>Amount added (ng/mL)</th>
<th>SQ 27,519 det’n (n = 6)</th>
<th>SO 28,555 det’n (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>43.4 ± 3.8</td>
<td>42.8 ± 2.2</td>
</tr>
<tr>
<td>400</td>
<td>421 ± 26</td>
<td>368 ± 30</td>
</tr>
<tr>
<td>800</td>
<td>784 ± 23</td>
<td>766 ± 37</td>
</tr>
<tr>
<td>4000*</td>
<td>3832 ± 111</td>
<td>3405 ± 159</td>
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</table>

*sample diluted tenfold prior to extraction
UTILIZATION OF THE HEATED NEBULIZER AND ION SPRAY INLETS FOR DRUG
METABOLISM STUDIES: A GENERAL STRATEGY FOR LC/MS ANALYSIS

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The utilization of atmospheric pressure ionization LC/MS techniques such as ion spray and heated nebulizer interfaces, both found on an API III triple quadrupole mass spectrometer, provide complementary tools for the identification of metabolites of novel therapeutic agents. The first technique, ion spray, produces gas-phase ions for those species which exist as ions in solution. The absence of heat makes this an ideal means of analyzing phase II metabolites, such as glucuronide and glutathione conjugates. However, since ion spray works best with flow rates of under 100 μL/min, the eluent from a conventional bore column must be split 10:1 or 20:1 to be compatible with the inlet. The heated nebulizer inlet uses flash evaporation of the LC eluent without flow splitting, followed by chemical ionization. For compounds which are not strongly ionized in solution and are thermally stable and volatile, this inlet offers an excellent means of analysis.

We have found that utilization of both the ion spray and heated nebulizer interfaces offer the best approach to the unequivocal molecular weight and structural determination of metabolites in a complex biological matrix. Even with the flow splitting necessary to utilize ion spray in conjunction with conventional bore columns, the technique produces intact molecular ions for glucuronide conjugates 10-15 times more intense than molecular ions produced by the heated nebulizer probe. However, for the detection and structural elucidation of thermally stable phase I metabolites, such as hydroxylated products, the heated nebulizer inlet is the technique of choice since all of the chromatographic eluent is directed to the mass spectrometer. This enables detection and characterization of low-level metabolites.

This dual-approach strategy is illustrated in the analysis of the metabolites of compound A, shown in Figure 1. A water/acetonitrile gradient, using 0.1% TFA, was used as the mobile phase with a 3 cm C18 cartridge column at a flow rate of 1.0 mL/min. 10 μL of bile obtained from a bile-duct cannulated monkey that had been dosed with this compound was injected for analysis. The TIC produced via ion spray is shown in Figure 2. The reconstructed ion chromatogram for the (M+H)+ ion of hydroxylated glucuronide metabolite, m/z 729, and its oxidation product to the keto/aldehyde glucuronide metabolite present at m/z 727, is shown in Figure 3. Good reconstructed ion chromatograms are produced for the conjugates, even though the eluent is split 20:1 prior to mass spectrometric analysis. However, analysis of the glucuronide conjugates by the heated nebulizer inlet produces drastically reduced ion currents, as shown by the reconstructed ion chromatograms depicted in Figure 4. The mass spectrum of peak A produced by ion spray shows an intact (M+H)+ ion at m/z 727; the mass spectrum produced by the heated nebulizer inlet (Figure 5) shows no molecular ion, only the aglycone at m/z 553.

On the other hand, since all the eluent is directed to the mass spectrometer, the heated nebulizer inlet produces higher signal intensity for thermally stable phase I metabolites. Figure 6 compares the reconstructed ion chromatograms, corresponding to dihydroxylated metabolites at m/z 569, produced by the ion spray and heated nebulizer inlets. The ion current produced by the heated nebulizer for these metabolites is about 20 times more intense than the ion current produced by ion spray. Thus, the heated nebulizer is preferable for phase I metabolites, due to the increased signal intensity.

The results outlined above have led us to apply a general strategy to the API LC/MS analysis of metabolites. First, one LC/MS analysis is carried out in full scan mode using the ion spray inlet. This analysis provides unequivocal molecular weight data, even for minor component glucuronide conjugates. The analysis is repeated in daughter ion MS/MS mode using the heated nebulizer interface. Daughter ion spectra are acquired for all the (M+H)+ ions of the phase I metabolites as well as the (M+H)+ ions of the aglycones produced via thermal degradation.
Electrospray Tandem Mass Spectrometry of Polyether Ionophores
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Polyether ionophores are fermentation derived biologically active compounds with a unique ability to form stable complexes with alkaline or alkaline earth cations. They exert their biological activity by catalyzing an electroneutral cation-proton exchange across cell membranes, moving as undissociated acids in one direction and as neutral complexes in the other. Their usefulness as cardiovascular drugs has been evaluated but their commercial success is due to their wide utilization as anticoccidiosis agents in broiler chickens and as feed efficiency enhancers in cattle and sheep.

Pneumatically assisted electrospray mass spectrometry of polyether ionophores yield several molecular ion adducts. A specific metal adduct molecular ion can be obtained by the selective addition of one salt to the HPLC mobile phase. This approach can aid molecular weight determination of unknown ionophores and the investigation of their metal ion preference. By allowing the selected ion monitoring (SIM) of any of several possible molecular ions, analytical methods can be rendered more specific and interferences from matrix can easily be avoided. For example, selected ion monitoring of salinomycin residues can be based on either of the following ions: m/z 773 (I+Na)+, m/z 789 (I+K)+, or m/z 883 (I+Cs)+, depending on the salt added to the mobile phase. Detection limits in the low ppb range have been demonstrated.
Collision induced dissociation of the molecular ions provides additional structural information and enhanced specificity for trace analysis. The CID daughter spectrum of the ionophore maduramicin is shown in Figure 1. The sodium adduct molecular ion is dissociated to two fragments. The minor daughter is consistent with the neutral loss of a CO2 molecule from the molecular ion. The most abundant daughter is apparently initiated by an intramolecular proton transfer from the carboxylic acid to the hydroxy group of the β-hemiketal, and then followed by the concerted losses of water and carbon dioxide to produce a polyether olefin (1). Mild CID conditions provide additional evidence for dimer formation. In the divalent ionophore lasalocid, the CID of its dimer ion (2I + 2Na - 1)+ at m/z 1225 resulted in only a single daughter ion (I+Na)+ at m/z 613.

Electrospray Mass Spectrometry: A Study on some Aqueous Solutions of Metal Salts


This work was undertaken to answer a fundamental question in electrospray: Is there a correlation between the electrospray mass spectrum and aqueous solution chemistry? Our initial attempt was centred on well characterized proteins such as myoglobin and cytochrome c (Roger Guevremont, K.W.M. Siu, J.C.Y. Le Blanc and S.S. Berman; Is the Electrospray Mass Spectrum of Proteins Related to their Aqueous Solution Chemistry? ASMS 91). However, even for these proteins, not sufficient information on their solution equilibria is available for a rigorous evaluation.

We have chosen to work with aqueous solutions of metal salts because much information exists and some of it has been critically evaluated. In this first round, we opted to prepare our samples by simply dissolving known quantities of metal salts in water and avoiding the addition of any acids, bases or organic solvents. Electrospray mass spectra of 1-3 mM aqueous solutions of some 30 metal salts, mostly metal halides and nitrates, have been recorded to date. Most spectra are dominated by hydrated clusters of the metal ion, metal salt complexes and metal hydroxides. For example, for cadmium iodide, the predominant ions observed were \( \text{Cd}(\text{H}_2\text{O})_n^{+} \), \( \text{Cd}(\text{H}_2\text{O})_n^{+} \) and \( \text{Cd}^{+} \) (Fig. 1). Typically, the most intense ions measured were those that had \( n = 1 \) or 2, and \( n = 5, 6, \) or 7 for singly and doubly charged ions, respectively.

Formation constants of a large number of metal anion complexes are known and have been critically evaluated [1]. We were able to calculate equilibrium concentrations of metal complexes in aqueous solution by entering these constants, the total metal and anion concentrations as well as pH into the Fortran program, COMICS [2]. Good correlation was observed between response and calculated equilibrium concentration for ions of the same charge and type. Fig. 2 shows results of some doubly charged ions that have been studied. Correlation between ions of different charge and type was relatively poor. This was believed to be caused by differences in the effect of electrospray on them. For example, the singly/doubly charged response was always larger than calculated singly/doubly charged concentration. This could be attributed to the large difference in solvation energies between singly and doubly charged ions.

For ions of the same charge and type, e.g., \( \text{Cd}(\text{H}_2\text{O})_n^{+} \) and \( \text{Cd}(\text{H}_2\text{O})_n^{+} \), excellent agreement was obtained between the mass spectrometric response and the calculated solution concentration when the total chloride/iodide ratio was varied from 20/1 to 1/20 under constant cadmium concentration (Fig. 3). Another example is the comparison between \( \text{Ag}(\text{NH}_3)_2^{+} \) and \( \text{Ag}(\text{NH}_3)_2^{+} \); very good correlation was obtained between their relative response and relative solution concentration when ammonia concentration was increased from 0.1 to 20 mM in a 2mM silver nitrate solution (Fig. 4).


A COMPARISON OF THE FOUR SECTOR MS/MS SPECTRA OF MOLECULAR AND QUASI-MOLECULAR IONS OF THE CYCLIC PEPTIDE VALINOMYCIN FORMED USING EI, CI, FAB AND FD

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Four sector mass spectrometry has been a successful tool in studying peptide ions of up to 2.5kDa, generally giving sufficient daughter ion mass accuracy to unambiguously assign peaks. To date, most work has employed FAB or LSIMS as the ionisation method. The present work follows an earlier study [1] in which the MIKES spectra of peptide ions formed by FD and FAB were compared. Dramatic differences were seen e.g. for the cyclic peptide beauvericin (RMM 783.4) the MIKES spectra of the [M+Na]⁺ ions formed by FD and FAB show different major fragmentation pathways. For the linear peptide bradykinin (RMM 1099.6) the MIKES spectra of [MH]⁺ ions formed by FAB and FD show great differences in the relative intensities of the fragment ions although the same fragments are seen.

The cyclic depsipeptide valinomycin (RMM 1110.6, structure shown in Fig. 1) was chosen for study since it is amenable to ionization by EI, CI, FAB and FD. The results are summarised below:

M⁺ ions are formed by field desorption (FD) and by electron impact (EI) using an electron energy of 70 eV. Both MS/MS spectra show the same dominant neutral loss of C₃H₇ involving a McLafferty rearrangement of valine (Val) or hydroxyisovaleric (HyV) acid residues. M⁺ ions, formed by EI fragment to a much greater extent which presumably reflects the much greater input of energy by the ionization process. A second dominant fragmentation forming [M-113]⁺ ions is seen in EI-MS/MS. This can be assigned to cleavage of the CH-O bond in the HyV or Lactic acid (Lac) residue and loss of Val-0 with transfer of two hydrogens to the charged fragment ie [M-(Val+O-2H)]⁺.

MH⁺ ions formed by either fast atom bombardment (FAB) or methane chemical ionisation (CI) give very similar MS/MS spectra. As with M⁺ ions, the spectrum is dominated by a single fragmentation, but in this case loss of 28 (presumably CO) is the major fragmentation instead of loss of 42 (C₃H₇) as seen for M⁺. MH⁺ ions formed by ammonia CI contain ~50% contribution from ¹³C isotopes of M⁺ resulting in an MS/MS spectrum containing ions from both EI and CI-MS/MS spectra. [M+NH₃]⁺ ions shows a major loss of NH₃ then similar fragmentation to [MH]⁺.

The most dramatic difference seen between similar adduct ions is for [M+Na]⁺ ions formed by FAB and FD (fig.1). FD-MS/MS gives a single dominant fragmentation of loss of 43 (C₃H₇), the isopropyl side-chain from Val and HyV residues. Much more extensive fragmentation is seen in the FAB-MS/MS spectra of [M+Na]⁺ ions, including major fragmentations forming [M+Na-(Val-Lac+O-2H)]⁺ and [M+Na-(HyVal)]⁺ ions. In addition, comparing the MS/MS spectra of [NH]⁺ and [M+Na]⁺ ions formed by FAB, the latter shows much greater fragmentation than the former, indicating that FAB ionisation results in more energetic [M+Na]⁺ ions than [NH]⁺ ions, possibly resulting in ring-opened structures and greater fragmentation. [M+K]⁺ ions formed by FAB show a similar degree of fragmentation to [M+Na]⁺ ions and the same two dominant fragmentations, although many different fragment ions are seen.
In conclusion, in the case of a large polyatomic ion such as valinomycin, the degree and nature of the fragmentation seen in the MS/MS spectra is dependent both on the ionization mode used and the type of adduct ion formed.

STUDIES OF THE EFFECT OF THE NATURE OF THE COLLISION GAS ON THE HIGH ENERGY CID OF PEPTIDES

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High energy collision induced decomposition (CID) in tandem double focussing mass spectrometers has been proved to be a successful method for sequencing peptides containing up to 25 amino acid residues [1]. The major fragment ion types that are present in the high energy CID of peptides have been established. Usually helium is used as collision gas in these experiments. There have been some conference reports which compared the CID of peptides obtained by helium to that obtained by heavier gases, such as argon, xenon and nitrogen. These investigations found indications that CID spectra obtained by heavier gases may contain significantly different features than those obtained by helium collision gas. We carried out investigations on the Concept IIHH tandem double focussing mass spectrometer in our laboratory in which we compared the qualitative and quantitative characteristics of the CID spectra obtained with light (He) and heavier (Ar, Xe) collision gases as a function of molecular weight of peptide precursor molecules. Three groups of peptides were investigated in order to take into account that peptides belonging to these different groups show different fragmentation behaviour from each other as has been observed previously by Biemann and coworkers [2]. The results of these investigations are summarized below.

1. Peptides containing arginine at the C terminal end

The CID spectra of this type of peptides show the strongest dependence on the nature of the collision gas. The Ar CID contains mainly abundant w_n and v_n ions and some y_n ions. When He was used as collision gas the intensity of the w_n and v_n ions decreased significantly, ca ten-fold or more. The spectra still contain some abundant y_n ions, but they generally do not form a complete series. These effects were stronger for peptides of greater molecular mass. An example of these effects is shown in Figure 1.

2. Peptides containing arginine at the N-terminal end

The He and Ar CID spectra of these peptides have similar features. They contain mainly a_n and d_n ions. The fragment ion intensities are also comparable for the light and the heavier gases. The main differences are in the relative abundances of the a_n and d_n ions. Argon CID generally produces significantly more abundant d_n ions than He CID, and it also generates d_n ions corresponding to amino acid residues which have aromatic side chains while He does not. Above ca 1500 dalton molecular mass the abundance of fragment ions in the He CID spectra decreases rapidly with molecular mass, while that in the Ar CID spectra remains relatively unchanged up to a molecular mass of 2500 dalton.

3. Peptides containing no basic amino acid residue

The CID spectra of these peptides are not affected significantly by the nature of the collision gas. These spectra contain mainly b_n and y_n ions. These ion types usually form a complete or near complete series some members of which have strongly reduced abundances.
The abundances of the fragment ions are fairly similar for both the light and the heavier collision gases.

Figure 1. Comparison of the high energy CID spectra of pancreastatin peptide fragment [33-49] obtained with He and Ar collision gases


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THE COMPLEMENTARY UTILITY OF ELECTROSPRAY TANDEM MS ON A TRIPLE QUADRUPOLE AND FAB TANDEM MS ON A FOUR-SECTOR INSTRUMENT

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Structural elucidation of unknown peptides and natural products presents unique mass spectrometric challenges. Although the high-energy CAD tandem MS proves to be the preferred technique in most cases, there are a number of situations where electrospray low-energy CAD may provide complementary useful information or even be the method of choice. Factors favoring the use of electrospray on a triple quadrupole are its excellent sensitivity, ease in rapid acquisition of many spectra from an infused mixture, versatility in terms of alternative scan modes, and simplicity of fragmentation for peptides (no side-chain cleavages and insignificant x- and z-type ions). These factors offset the method's lower resolution, poorer mass accuracy, inability to distinguish isomeric amino acids or establish disulfide-bond connectivity, and frequently incomplete sequence information for peptides when compared to high-energy CAD on a four-sector instrument.

The limit of analysis, defined as the minimum amount of sample for which a product-ion spectrum could be recorded with essentially the same analytically useful structural information obtainable from unlimited sample, was determined to be about 500 fmol by ESI/MS/MS (Fig. 1). This represents about 10-50 fold improvement over what we can do by FAB/MS/MS on the four-sector mass spectrometer with conventional electron multiplier detection. The mass range for CAD does not appear to be any better on the four-sector than on the triple quadrupole using ESI. Nearly complete sequence information was obtained by both methods for adrenocorticotrophic hormone fragment 18-39 \((M_r = 2464)\) (for ESI see Fig. 2), neither instrument was able to obtain fragmentation data from a peptide of \(M_r = 2307\), while only ESI/MS/MS was able to obtain any useful sequence data from a peptide of \(M_r = 1996\), and that, only from the triply-charged precursor (Fig. 3). In the case of a tryptic peptide of \(M_r = 1498\), ESI/MS/MS yielded more interpretable data and slightly better sequence coverage, while the four-sector data was able to clearly differentiate leucine isomers.

With proper technique, it should be possible to acquire informative MS/MS spectra consuming less than 5 pmol of sample.

Sequence information for ACTH derived from the doubly-charged parent complements and overlaps with that derived from the triply-charged parent (not shown). Together they provide sequence information for all but the N-terminal arginyl-proline. The presence of arginine is suggested by the immonium ion - ammonia at \(m/z\) 112.
Spectra of enzymatic digests of proteins are considerably more complicated when acquired by electrospray versus FAB ionization due to multiple charging. Nevertheless, the electrospray method lends itself to continuous infusion of small amounts of sample (<25 pmol/μl) at low flow rates (1 μl/min), so that MS/MS of all intense ions in the spectrum of a protein digest mixture can be acquired rapidly and in automated fashion with relatively little sample consumption. The resultant data may not equal the four-sector tandem mass spectra in terms of sequence completeness or structural detail, but a portion of the sequence is nearly always readily available. In the mapping of proteins of known structure, this ability allows rapid sequence confirmation and localization of post-translational modifications. In the case of sequencing peptides of unknown structure, the simplicity of the results and, more importantly, the clarity with which sequence direction can be determined, provide a convenient springboard for the interpretation of the more detailed, and more complex high-energy CAD data.

An example of the importance of high-energy CAD for detailed structural analysis is its ability to establish disulfide-bond linkages. High-energy CAD MS/MS on selected peaks in an unpurified thermolysin digest of TGF-α produced clear evidence that all three disulfide bonds were correctly formed. This is possible because high-energy CAD of disulfides produces unusually intense disulfide cleavage ions (Fig.4). The same is apparently not true for low-energy CAD. In contrast, an experiment that is particularly well-adapted to ESI/MS/MS on a triple quadrupole is the use of parent scans from the oxonium ion of N-acetyl hexosamine for specific detection of glycopeptides during LC/MS/MS of protein digests (see Huddleston, M. J. et al, this volume).
Tandem Mass Spectrometry (MS/MS) of Lithium-attachment Ions from Polyglycols.

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Earlier work from our laboratory investigated in some detail the MS/MS behavior of MH+ and MNa+ ions from linear poly(ethylene glycol), PEG, and poly(propylene glycol), PPG. The MH+ ions fragmented readily under low energy collisional activation (~50 eV), but the product ions formed were of relatively low mass; hence these were of limited value for chemical structure elucidation. The MNa+ ions, on the other hand, while generally quite intense, were found to be very stable towards cleavage of C-O and C-C bonds. Only very weak sodiated organic product ions were formed, even at a relatively high translational energy (3 keV), with collision gas air. Thus it was found that neither MH+ nor MNa+ ions were optimal for practical MS/MS analysis of polyglycols.

We have now carried out a detailed study of the MS/MS behavior of three glycol polymers (linear PEG, linear PPG, and an ethoxylated fatty alcohol) using a Finnigan MAT 95Q hybrid mass spectrometer of BEoQ geometry. Ionization was provided by fast atom bombardment (FAB). Collisional activation was carried out in the "collision octapole" at a relatively low translational energy (50 eV), with collision gas air. A principal thrust of this study was to investigate the analytical utility of MS/MS of lithium-attachment polyglycol ions. It was found that MLi+ ions provide a number of important advantages for practical MS/MS analysis as compared to the use of MH+ or MNa+ ions. First, MLi+ parent ions for polyglycols are generally much more intense than the corresponding MH+ ions. Second, MLi+ ions dissociate at low collision energies (~50 eV), in contrast to the much more stable MNa+ ions. Third, product ions are generally formed over the entire mass range for low molecular weight polyglycols. This feature is especially useful for analytical purposes, i.e., the chemical structure analysis of unknown polyol chemicals.

The MS/MS fragmentation mechanisms for MLi+ ions have been studied in some detail. Deuterium-labeled polyglycols were used to assist in this investigation. Product ion scans from MLi+ of linear PEG decamer (m/z 465) and PPG octamer (m/z 489) are attached as Figures 1 and 2, respectively. One thing that is immediately clear is that reasonably intense, characteristic fragment ions are observed throughout the entire mass range. Four ions series are marked (A, B, C, D). The most intense product ions are in the A series; these are lithiated, linear glycol oligomers (R = H for PEG and CH3 for PPG):

\[
\text{Li}[\text{H-(-O-CH}- \text{CHR}-)_{n} \text{OH}]^{+} \quad \text{PEG: m/z 44n + 25; PPG: m/z 58n + 25.}
\]

These ions are formed by internal hydrogen transfer reactions which likely proceed through six-membered ring intermediates (see scheme below for PEG). It is likely that lithium plays some specific role in facilitating these charge-site initiated rearrangements.

The B and C series ions are logically formed via charge-remote fragmentations (analogous to those described previously for MNa+ ions):  

- B series. Li[\text{H-(-O-CH}- \text{CHR}-)\text{-O-CH=CHR}]^{+} \quad \text{PEG: m/z 44(n+1) + 7; PPG: m/z 58(n+1) + 7.}

- C series. Li[\text{OCH-CHR-(-O-CH}_2\text{-CHR-)}_{n}\text{-OH}]^{+} \quad \text{PEG: m/z 44(n+1) + 23; PPG: m/z 58(n+1) + 23.}

The low mass D series ions are radical cations containing lithium. These evidently form via homolytic bond cleavages near chain ends.

The third compound studied was Neodol 25-7, an ethoxylated fatty alcohol:

\[
\text{C}_{m+2}\text{H}_{2m+1}\text{-O-(C}_2\text{H}_4\text{-O)-}_n\text{-H MW 14m + 44n + 18.}
\]

The pattern of MLi+ product ions is very similar to that observed for PEG (Figure 1). In fact, the four ion series noted for PEG (A, B, C, D) are also prominent for Neodol 25-7; these series are all derived from the PEG end of the molecule. A fifth prominent ion series (A') is derived from the alkyl end of the molecule: Li[C_{m+2}\text{H}_{2m+1}\text{-O-(C}_2\text{H}_4\text{-O)-}_n\text{-H}]^{+} \quad \text{m/z 14m + 44n + 23. In summary, MS/MS analysis of MLi+ ions from the ethoxylated aliphatic polymer (Neodol) proved to be quite useful for chemical structure elucidation. It appears that the lithiation / tandem mass spectrometry combination will be a very helpful tool in analyses of selected polar, low molecular weight polymeric materials.}

\[
\text{OCH-CH}_2\text{-O-(-O-CH}_2\text{-CH}_2\text{-O-)}_{n}\text{-OD}
\]

\[
\text{OCH-CH}_2\text{-O-(-O-CH}_2\text{-CH}_2\text{-O-)}_{n}\text{-OD}
\]

Product Ion Scan (FAB-MS/MS) of M Li+ 465 (ethylene glycol decamer).

Product Ion Scan (FAB-MS/MS) of M Li+ 489 (propylene glycol octamer).
IMPROVED DETECTION LIMITS FOR MS/MS OF DNA-PAH ADDUCTS

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Covalent bonding of chemical carcinogens to DNA is likely the first step in tumor formation. Polycyclic aromatic hydrocarbons (PAH) can be converted into electrophilic intermediates, which ultimately react with DNA, by either one-electron oxidation to form radical cations or by monoxygenation to produce diol epoxides. To ascertain the biological significance of these mechanisms, the structure of DNA-PAH adducts must first be determined.

FAB combined with tandem mass spectrometry can differentiate between isomeric forms of adducts formed between benzo[a]pyrene (BP) and DNA [1]. With direct probe introduction and scanning of MS-II (an electrostatic analyzer), the detection limit for these adducts is 100 ng on a Kratos MS-50 triple analyzer. Although this level is acceptable for in vitro studies where experimental parameters can be scaled up, detection limits in the pg range are required for in vivo studies of biological systems. These experiments are necessary to determine the actual significance of a proposed mechanism.

Improvement in both detection limit and in the quality of CAD spectra is obtained by using a VG ZAB-T, a Mattauch-Herzog like four-sector tandem mass spectrometer. Shown in Figure 1 are the CAD spectra of adducts formed between adenine (Ade) and dimethyl-benzanthracene (DMBA). The enhanced resolution of the four sector instrument allows differentiation between adducts formed at the C7-CH3 (upper) or C12-CH3 of DMBA (lower) by comparison of the relative abundances of two major fragment ions. CA of the Ade adduct involving the C7-CH3 of DMBA produces a more abundant ion of m/z 239 relative to 240; CA of the adduct formed at the C12-CH3 position of DMBA results in a more abundant ion of m/z 240. Because the resolution of the electrostatic analyser that serves as MS-II on the Kratos MS-50 Triple Analyzer is < 250, these two adducts could not be distinguished based upon their CA spectra of (M + H)⁺.

An example of the improvement in sensitivity is shown in Figure 2. The upper section is the CAD spectrum of 20 ng of a benz[a]pyrene adduct of guanine (Gua). It compares favorably with a reference spectrum of 2 ug of the adduct (lower section). This improvement in sensitivity may principally be due to the use of a Cs⁺ SIMS gun, operated at 25 keV above source potential, on the ZAB-T instead of a 6 keV FAB gun as outfitted on the MS-50 TA. Even with this improvement, the detection limit is still high. The spectrum of 20 ng contains several noise spikes of comparable intensity to the signals for the fragment ions, even though 15 scans were averaged to produce the CAD spectrum. It is evident that the means of detection is a limiting factor in the MS/MS analysis.

The ZAB-T is equipped with a 15 cm photodiode array for simultaneous detection over a variable mass range. The use of the array improves the detection limit for direct probe introduction of the BP-Gua adduct by two orders of magnitude. Figure 3 contains the partial CAD spectra obtained on 2 ng and 200 pg of the adduct. The spectra are the result averaging together several 3 second array integrations. The ion of m/z 248 is a matrix interference and points out that sensitivity is now limited by the ionization process rather than by the detection method.

Continued improvement in sensitivity is dependent on better parent ion production, both in efficiency and in selectivity. Since we have successfully obtained CAD spectra on 1 to 10 picomole samples of peptides by using continuous flow FAB, we feel that the technique holds promise for the analysis of PAH-DNA adducts. This technique is, however, still limited by matrix interferences, albeit at lower levels. We are, therefore, pursuing the use of other ionization methods, such as field desorption or laser desorption, to minimize the effects of chemical noise on detection limits.

Figure 1. CAD spectra of DMBA-C7-CH2-N3Ade (upper) and DMBA-C12-CH2-N7Ade (lower).

Figure 2. CAD spectra of 6-BP-N7Gua obtained using a single point detector; upper spectrum of 20 ng, lower spectrum of 2 ug.

Figure 3. CAD spectra of 6-BP-N7Gua using an array detector; upper spectrum of 2 ng, lower spectrum of 200 pg.
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DETERMINATION OF ISOTOPIC ENRICHMENTS USING TANDEM MASS SPECTROMETRY

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The significance and utility of the isotope patterns observed in certain tandem mass spectrometric analyses have been appreciated for some time. Todd et al. (1), for example, recognized that the isotope patterns observed in the product ion spectra derived from precursor ions incorporating multiple isotopes reflected the elemental compositions of the precursor ions. Bozorgzadeh et al. (2) similarly noted that the elemental compositions of precursor ions can be determined from a comparison of the product ion spectra of $m_1^+$ and $(m_1 + 1)^+$. The particular value of observed isotope patterns in precursor ion spectra and constant neutral loss spectra was illustrated by Singleton et al. (3). The principles involved may be explicitly stated as follows. For a single fragmentation process, (i) a precursor ion scan defines the isotopic composition of the neutral fragment lost, and (ii) a constant neutral loss scan defines the isotopic composition of the product ion detected. These principles have hitherto been put to little practical use. Here we illustrate their application in three areas and indicate the need for caution in interpretation when isotope patterns are distorted by the occurrence of oxidation or reduction processes in concert with the ionization step.

(a) Determination of isotope enrichment in labeled compounds. Two particular advantages accrue from the application of tandem MS in this area. First, isotope incorporations may be determined even when the analyte is present in a mixture sufficiently complex to obscure the conventional mass spectrum. Second, judicious choice of tandem MS scans permits the determination of isotope enrichments in specific locations in the analyte structure. A simple example is provided by $[^3H_2]$platelet activating factor (1-O-[[H]4]hexadecyl-2-acetyl-sn-glycero-3-phosphocholine). Metastable decomposition of the protonated species (m/z 528) yields principally m/z 184, corresponding to the phosphocholine headgroup. A scan of precursors of m/z 184 then defines the isotopic composition (with enrichment of deuterium) of the substituted glycerol moiety whereas a constant neutral loss scan of 344 Dalton reflects the natural isotopic composition of the phosphocholine.

(b) Isotope patterns as an aid to spectral interpretation. During FAB/tandem MS analysis of the natural glutathione conjugate, leukotriene C4, scanning of precursors of m/z 308 (presumptively representing protonated glutathione) yielded an ion abundance pattern in the [M+H]^+ ion region (m/z 626-628) which reflected the natural isotopic composition of the lipid sub-structures of the precursor molecule. This correspondence thus substantiates the proposed fragmentation assignment. Observation of signals at m/z 625 and 624, however, provided evidence for the occurrence of one- and two-electron oxidation processes occurring in concert with FAB ionization. Distortion of the apparent isotope pattern attributable to reduction processes has been observed during FAB/tandem MS analysis of the glutathione conjugate of acetaminophen (Figure 1). When the FAB liquid matrix was glycerol, scanning of precursors of m/z 182 revealed the expected predominance of m/z 488, but m/z 490 was significantly more abundant than expected on the basis of naturally occurring abundance. When trifluoroacetic acid (TFA) was added to the liquid matrix, the relative abundances of m/z 488-490 corresponded to the expected isotope pattern, in keeping with the findings of Visenti et al. (4) concerning the suppression of reduction in the FAB matrix following addition of TFA.

(c) Applications in drug metabolism studies. We have previously described (5) the joint application of the twin ion technique and tandem MS in metabolism studies. Here we emphasize the interpretative value of isotope patterns imposed by the use of derivatization reagents of defined isotopic composition. FAB MS of the bis-methyl ester of S-butyl glutathione, prepared using methanol/[^3H_3]methanol (1/1), gave the spectrum shown in part in Figure 2. Since the analyte is a bis-carboxylic acid, a 1:2:1 isotope pattern is observed in the molecular ion region. Constant neutral loss scanning of either 143 or 146 Dalton, corresponding to loss of the methylated gamma-glutamyl moiety, collapses the isotope pattern to a 1:1 doublet (Figure 3). This strategy has been applied in the screening for glutathione conjugates derived from 2-furamide in rat bile, where the expected (and observed) pattern is complicated (but in a predictable fashion) by the use of a 1/1 mixture of unlabelled and $[^3C_1]$-labelled xenobiotic in the metabolism experiment.

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References

Figure 1

Figure 2. Conventional FAB mass spectrum (MH⁺ region) of S-butyl glutathione bis-methyl (²H₀/²H₃) ester.

Figure 3. Tandem MS analyses of S-butyl glutathione bis-methyl (²H₀/²H₃) ester with constant neutral loss scanning of 143 Da (A) and 146 Da (B).
MS/MS STUDIES OF PROTONATED AND ALKALI CATIONIZED ETHYLENE GLYCOLS

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Fragmentation pathways of alkali cationized molecules frequently differ from those of the related protonated molecules. To explore these differences, we studied the metastable and collisionally activated dissociation, CAD, mass spectrometry of polyethylene glycol dimethyl ethers. These compounds, commonly referred to as the glymes, have the general formula \( \text{CH}_3(\text{OCH}_2\text{CH}_2)_n\text{OCH}_3 \). We investigated the protonated and cationized glymes of various sizes ranging from triglyme \((n=3)\) to nonaglyme \((n=9)\) and where the cations were \( \text{Li}^+, \text{Na}^+, \text{K}^+, \text{and Cs}^+ \).

The experimental conditions were as follows. MS/MS: B/E Link scan technique on a VG 7070E double focusing mass spectrometer. Ionization: FAB. Ion energy was 5 kV. CAD: Collision gases were argon or air, with parent ion attenuation at 50%. Alkali cations were from iodide salts.

Figures 1 and 2 show the metastable and CAD mass spectra of the \((M+H)^+\) ion of tetraglyme, respectively. In both spectra the fragmentation is dominated by cleavage of the C-O bond accompanied by a hydrogen rearrangement. The daughter ion yield under metastable conditions is 2%. Under CAD, the total yield of daughter ions doubled to 4.2%, this increase arising mainly from the enhancement of the \(m/z\) 103 and 59 ions. For comparison, Figures 3 and 4 show the CAD spectra of penta- and nonaglymes, respectively. The dominance of low mass peaks is clearly observed in these two spectra, a similar pattern as seen in the CAD of tetraglyme (Fig 2).

We believe that the main reasons for this dominant low mass pattern involve ion internal energies. Parent ions with a relatively high degree of internal energy would tend to undergo sequential fragmentations to smaller granddaughter ions. We note that the ion \(m/z\) 59, though a minor peak in the metastable spectrum, appears as the base peak in the prompt FAB spectra of the glymes (not shown). This suggests that the protonated glymes that are selected for metastable scanning, though energetically cooler than those undergoing prompt fragmentation, have sufficient internal energies to undergo sequential fragmentation. Collisional activation increases the chances of sequential fragmentation and the CAD results support this notion; the dominant low mass pattern shifts towards smaller fragments (i.e. \(m/z\) 103 and 59). Other factors for the low mass pattern may arise from the influences of dichromination of the proton within the glyme. Ion-dipole interactions between the proton and the \(-\text{CH}_2\text{OCH}_2-\) groups have been postulated by Kebarle.1 Stronger ion-dipole interactions occur more frequently towards the ends of the glyme chain where there can be optimal ion-dipole alignment (Fig. A). This ion-dipole stabilization may play a role in the fragmentation processes but it remains unclear what the influences of dichromination would be.

Cationized glymes exhibit different behavior from the protonated glymes. (We discuss here the sodium adducts; the other alkali cation adducts exhibited similar results.) Figure 5 shows the metastable spectrum of the \((M+Na)^+\) ion of tetraglyme. The spectrum is weak and the daughter ion yield is 0.08 %, lower than the protonated tetraglyme by a factor of twenty. This data suggests that the \((M+Na)^+\) ion is energetically cooler than the protonated ion. Coordination of the alkali cation by the \(-\text{CH}_2\text{OCH}_2-\) dipoles, resulting in a crown ether/sodium ion-like complex (formed in solution, prior to FAB desorption) (Fig. B) can serve to stabilize the \((M+Na)^+\) ion.

Upon collisional activation of the \((M+Na)^+\) of tetraglyme (Fig. 6), the daughter ion yield increased by a factor of seven over the yield from the pure metastable fragmentation. CAD has also dramatically increased the number of fragment ions observed. This high degree of uniform fragmentation was also observed for the larger nonaglyme \((M+Na)^+\) ion (Fig. 7).

Although the absolute daughter ion yields from CAD are smaller for \((M+Na)^+\) than \((M+H)^+\) ions and the CAD spectra from both of these ions are distinct, these differences diminished upon increasing the percent attenuation of \((M+Na)^+\) parent ions by the collision gas. In going from fifty to ninety percent attenuation, we found that the CAD daughter ion yield of the \((M+Na)^+\) of tetraglyme approaches the yields found for protonated tetraglyme and the fragmentation pattern becomes dominated by lower mass daughters, reminiscent of the dominant low mass peaks from CAD of the \((M+H)^+\) ion. The ion internal energies therefore appear to be the main properties influencing the nature and degree of fragmentation.

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**Fig. 1** Metastable of (M+H)^+ of Tetraglyme

**Fig. 2** Collisionally Activated Dissociation of Tetraglyme (M+H)^+

**Fig. 3** CAD of Pentaglyme

**Fig. 4** CAD of Nonaglyme

**Fig. 5** Metastable of (M+Na)^+ of Tetraglyme

**Fig. 6** Collisionally Activated Dissociation of Tetraglyme (M+Na)^+

**Fig. 7** CAD of (M+Na)^+ of Nonaglyme

**Fig. A**

**Fig. B**

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Steranes are a class of organic compounds called biomarkers that retains the basic carbon skeletons of biological compounds through diagenesis and maturation during burial. The distributions of biomarkers can be used to assess source input, age, maturity, biodegradation and depositional environment of source rocks and oils. They can also be used for oil/oil and oil/rock correlation studies.

Conventionally, GC/MS is used to monitor the distributions of two principal biomarker types, namely, steranes and triterpanes. However, due to interferences from coeluting components, particularly in the case of steranes, certain GC/MS measurements can be difficult. Compromises are usually taken for determining the distributions of various types of steranes, such as the use of m/z 217 chromatogram for regular steranes, m/z 218 for isosteranes and m/z 259 for diasteranes. GC/MS/MS, which links molecular ion and characteristic fragment ions, provides a clean separation between homologous series of coeluting steranes (see below), eliminating many of the compromises necessitated by GC/MS for characterizing sterane distributions.

The use of normal geometry tandem sector instruments with a multiple reaction monitoring (MRM) technique was initially reported in the literature for the parent GC/MS/MS experiments[1]. Due to rather high kinetic energies (in keV) of daughter ions reaching the detector, reasonable sensitivities can be easily obtained without re-tuning the instrument. The use of a tandem quadrupole MS/MS, on the other hand, requires re-tuning of the instrument to achieve optimal sensitivities for the parent experiments. However, tandem quadrupole yields chromatographic peaks with baseline resolution while MRM yields raised baseline due to inadequate energy resolution inherent with a tandem sector instrument.

We used a Finnigan TSQ-46 tandem quadrupole instrument for the GC/MS/MS parent experiments. The first quadrupole (Q1) was used to monitor the molecular ions while the third quadrupole (Q3) was set to allow only the m/z 217 ion to pass through. The middle quadrupole was used for collimation. Figure 1 shows typical GC/MS/MS chromatograms for steranes in a phosphatic marine-source rock. The top four traces (A through D) are the selective GC/MS/MS measurements between the molecular ions and the m/z 217 ions for the C27 through C30 steranes, respectively. It can be seen that the GC/MS/MS technique can determine distribution of diasteranes, isosteranes and regular steranes for each carbon homologue without any interference due to coelution. The bottom trace (E) is the Integration of Traces A through D, which shows a similar elution pattern to that observed by GC/MS when monitoring only the m/z 217 ion.

The 217 ions observed in GC/MS and GC/MS/MS are the products of fragmentation from the molecular ions in different time frames. The m/z 217 ions detected by GC/MS occur in the ion source with fragmentation rates faster than $10^{-6}$ seconds, while those by GC/MS/MS in the region between the Q1 and the Q3 with rates of on the order of $10^{-5}$ seconds. However, for steranes of the sample set we studied both techniques yield highly comparable results. Figure 2 shows similarity by comparing a m/z 217 chromatogram from GC/MS with an integrated 217 chromatogram from GC/MS/MS measurements of a carbonate marine-source rock. Thus, the value of GC/MS/MS in the analysis of steranes does not lie in the ability to better determine routine sterane parameters employed in petroleum exploration applications, but rather in the ability to characterize steroidal hydrocarbon distributions which cannot be adequately determined by conventional GC/MS. Two of such opportunities were identified: (1) the ability to characterize distribution of C30 steranes, a marine-source indicator, and (2) the ability to characterize A-ring methyl steranes[1]. Neither of these compound classes, which carry important geochemical information regarding organic matter sources and facies, can be adequately measured using GC/MS.
REFERENCES:

FIGURE 1
TANDER QUADUPOLE YIELDS CHROMATOGRAMS WITH BASELINE RESOLUTION

FIGURE 2
A WEALTH OF GEOCHEMICAL INFORMATION CAN BE OBTAINED FROM THE STEMN ELUTION PATTERN SHOWN IN THE CHROMATOGRAMS

FIGURE 3
COMPARABLE RESULTS ARE OBTAINED BY GC/MS AND GC/MS/MS
Time-of-Flight mass spectrometry (TOF-MS) has numerous advantages for the analysis of biomolecules, including virtually unlimited mass range (e.g., 274,800 Da) [1], high sensitivity (multichannel detection, high transmission), high speed, medium resolution (>20,000 demonstrated) [2], and relatively low cost. However, its capabilities for tandem MS have received little attention. Here, we describe a method for surface-induced dissociation (SID) [3] using an in-line collision device with a reflectron TOF instrument. We also demonstrate a novel method for combining matrix-assisted laser desorption [1] with continuous aqueous introduction for interfacing liquid-phase chromatographic techniques, such as capillary electrophoresis, to MS.

Ions from volatile samples are formed using 266 nm multi-photon ionization (MPI). For non-volatile biomolecules, aqueous sample solutions are introduced through a capillary directly into the ion source of the mass spectrometer; evaporative cooling results in a continuous flow of "ice" at the end of the capillary. Ions are laser desorbed from the ice matrix either directly (1.9 μm; 266 nm), or with two laser pulses (1.1, 1.9 μm neutral desorption; 266 nm MPI) from a single Nd:YAG laser with a double pulse option. For SID, ions are made to undergo collisions with a retractable stainless steel surface in the reflectron mirror. Collision energies are varied by adjusting both the position and voltage of the surface.

Ions are mass selected for dissociation using a pulsed deflection plate after the ion source; unit mass resolution at m/z 200 is obtained (Figure 1). This resolution increases with \( \sqrt{m} \) but is ultimately limited by the initial kinetic energy spread of the ions from the source as well as the rise time (and voltage) of the deflection pulse. Collision energies of the selected precursor ions can be continuously varied from 0 eV up to their acceleration energy. Most ions show extensive fragmentation with collision energies approaching 200 eV; above this, neutralization increases significantly, and desorption of secondary ions from the collision surface starts to occur. A typical breakdown graph of SID fragmentation of methyl-anlsole (Figure 2a) shows the expected fragmentation for this compound (80 eV SID spectrum Figure 2b). Daughter ion resolution of ~70 at m/z 100 (FWHH) is obtained with collision energies <100 eV.

![Figure 1: 266 nm MPI spectrum of Br-naphthalene (left), and with parent ion selection (m/z 208, right). Molecular ion region shown.](image)

Higher energy collisions produce extensive dissociation of even very stable ions, such as the molecular ion of phenanthrene (Figure 3). Surprisingly, abundant pick-up of species as large as C_{6}H_{9.4} by the molecular ion from the surface is observed. The source of this is most likely polyphenyl-ether diffusion pump oil used in our instrument. We observe no measurable shift in our flight times for these species, indicating that this process takes place spontaneously on the surface (<100 ns). Recent theoretical calculations indicate that such reactions can result from a single surface bounce (<1 ps) [4]. Fragmentation appears to be more extensive than that obtained by Cooks and coworkers [3] at the same collision energies, consistent with higher internal energy deposition obtained with our near normal collisions. Overall SID efficiencies of 7 - 15% are observed for most small molecules with 20-100 eV collisions. Preliminary SID spectra of peptides (Figure 4) indicate that efficiencies Preliminary SID spectra of peptides indicate that efficiencies of 30 - 50% are possible for these larger molecules.
Figure 2: SID breakdown graph for methyl-anisole (left). 40 eV SID spectrum (right)

Figure 3: 120 eV SID spectrum of phenanthrene molecular ion.

Figure 4: ~50 eV (top) ~200 eV (bottom) SID spectrum of insulin molecular ions formed by 266 nm laser desorption from a continuous flow ice/nicotinic acid matrix.

Broad Spectrum Analysis of Alcohols for Delineating Plasticizer Contamination in Soils and Surface Water

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In support of a monitoring program to delineate the extent of contamination by plasticizer at an industrial site a rapid analytical method was required for indicator compounds. GC/MS analysis employing EPA Method 8270 was not suitable as a single test for fingerprinting some sources. This was due in part to plasticizers containing multi-component mixtures of coeluting phthalate esters and adipates with poor diagnostic mass spectra.

The plasticizers investigated were suspected to contain various blends of: di(2-ethylhexyl)phthalate; tri(2-ethylhexyl)trimellitate; diisodecyl adipate isomers; diisodecyl phthalate isomers; diundecyl phthalate and dioctyldecyl phthalate. Preliminary tests conducted indicated a linear correlation between the levels of these components with detectable levels of C₇-C₁₁ alcohols in contaminated samples.

Indicator Compounds

This paper describes a GC/MS/FID strategy employing selected alcohols as indicator compounds for plasticizer contamination in soils and surface water. The indicator compounds selected for analysis occur as complex alcohol mixtures arising from the manufacturing process.

For example, the GC/MS total-ion profile of Blend #1 contained at least 8 isodecanol isomers. Another source of plasticizer, blend #2, coded as 810EE contained a mixture of 1-octanol and 1-decanol. A third blend contained a single component, 2-ethylhexanol. Conceptually, contamination of soil or surface water arising from these point sources could be distinguished from each other using the diagnostic alcohols as indicator compounds.

The analytical strategy selected for this monitoring program utilized a simple carbon disulfide solvent extraction procedure with off-line GC/FID quantitative analysis. GC/FID conditions were established to permit diagnostic identification of the three sources of plasticizer. Electron impact GC/MS analysis was reserved for secondary confirmations of phthalate esters as required. This strategy was required to: (a) reduce the response time between sampling and analysis and (b) minimize the cost of extensive analyses.

Extraction Procedures

Soil samples (10g) in 20 mL scintillation vials were agitated in water (8mL) to form a slurry which was extracted using carbon disulfide (5mL) for 5-10 minutes. The resulting mixture was allowed to settle for 10 min and 1 µL of the carbon disulfide fraction (middle layer) was analyzed directly without further cleanup. Poor recoveries below 5% were obtained using water only as the extraction medium. For some soils with noted organic matter, centrifugation was necessary to break up emulsions and facilitate separation of the carbon disulfide fraction from the extraction mixture. QA/QC data were obtained for the recovery of blends #1 - 3 using matrix spikes of soil samples covering the duration of the monitoring program. The recovery (for fortified levels at 40-300 µg/g) and method detection limits obtained for soil samples were 30-60% and 1 µg/g respectively.

Water samples (125 mL) were extracted in the sample containers with carbon disulfide (10 mL) for 5-10 minutes. The resulting mixture was allowed to settle, the carbon disulfide fraction transferred to a 20 mL scintillation vial and 8 mL of water added to suppress evaporation of the solvent. QA/QC data were obtained for the recovery of blends #1 - 3 using matrix spikes of water samples covering the duration of the monitoring program. The recovery (for fortified levels at 10-1000 µg/L) and method detection limit determined were 65-97% and 0.1 mg/L respectively.

Instrumental Procedures

The GC/MS/FID conditions utilized a Hewlett Packard 5996 GC/MSD equipped with TARGET software and a UNIX Chem Station. All confirmations were based on retention time data and electron impact data obtained for commercial standards.
of the indicator alcohols. GC/MS experimental conditions were established as per Method 8270 guidelines: 30m DB-5 column, i.d. 0.33mm, film thickness 0.25μm, helium carrier gas 1 mL/min, injector 280°C, transfer line 300°C, temperature program 150°C/5min 8 10°C/min - 280°C/7min. Off-line GC/FID conditions utilized a Shimadzu GC equipped with a 30m nukol column, i.d. 0.53mm, film thickness 0.5μm, helium carrier gas 6 mL/min, isothermal 120°C.

Quantification of the isodecanol isomers, blend #1, was performed using external standards and area summation of the isomers. Identification of real samples was facilitated by the diagnostic GC/FID fingerprints and GC/MS confirmations as required. Likewise, analysis of blend #2 in real samples was based on the presence of both 1-octanol and 1-decanol at relative concentrations of 1:1. GC/MS confirmation of the single alcohol in blend #3 was performed as required.

Conclusion
The GC/MS/FID strategy employing alcohols as indicator compounds provided diagnostic fingerprints of point sources. The strategy was found to be cost effective for delineating plasticizer contamination in a monitoring program. Actual samples from contaminated sites contained levels up to 1000 ppm. Appropriate dilutions were deemed necessary to minimize GC/MSD downtime and reduce the response time between sampling and analysis.

Acknowledgements
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FRAGMENTIONS PRODUCED BY ION SOURCE REACTIONS
IN THE METHANE NEGATIVE ION CHEMICAL IONIZATION
MASS SPECTRA OF PHTHALATES

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Negative ions appearing at m/z values greater than M' are frequently observed in methane negative ion chemical ionization (NICI) mass spectra. Excluding ions produced by reactions involving neutral or ionized forms of \( \text{O}_2 \) or \( \text{H}_2\text{O} \), these ions have been attributed to radical-molecule or surface-assisted reactions that occur prior to electron capture ionization. In addition to ions appearing at m/z values above M', a few examples of fragment ions produced by such reactions have appeared in the literature.\(^1\)

The methane NICI mass spectra of phthalates show unusual fragment ions that correspond to \([\text{M-C}_n\text{H}_{2n-1}]^-'\) and \([\text{M-OC}_n\text{H}_{2n-1}]^-'\). For example, ions at \([\text{M-14}]^-'\) and \([\text{M-30}]^-'\) are observed in the NICI mass spectrum of dimethyl phthalate (see Figure 1). These ions are of interest because they can be used to differentiate ortho, meta, and para alkyl esters of phenyl dicarboxylic acids; however, the ions are unusual because their formation requires either a hydrogen addition or intramolecular hydrogen transfer.

The \([\text{M-14}]^-'\) and \([\text{M-30}]^-'\) fragment ions from dimethyl phthalate have been studied using deuteromethyl phthalate and \( \text{CD}_4 \) reagent gas. The spectrum of deuteromethyl phthalate shows ions that correspond to \([\text{M+H-CD}_4]^-'\) and \([\text{M+H-OCD}_3]^-'\) (see Figure 2A), indicating that a hydrogen addition has occurred, not an intramolecular hydrogen transfer. Using \( \text{CD}_4 \) as the reagent gas, the spectrum of dimethyl phthalate shows that the added hydrogen originates from the methane buffer gas (see Figure 2B). The spectra of dialkyl phthalates with longer alkyl groups \((n = 2-10)\) also show the \([\text{M+H-C}_n\text{H}_{2n-1}]^-'\) and \([\text{M+H-OC}_n\text{H}_{2n-1}]^-'\) ions. As the number of carbons increase, the \([\text{M+H-C}_n\text{H}_{2n-1}]^-'\) and \([\text{M+H-OC}_n\text{H}_{2n-1}]^-'\) relative abundance decreases.

Several mechanisms could explain formation of the \([\text{M+H-C}_n\text{H}_{2n-1}]^-'\) and \([\text{M+H-OC}_n\text{H}_{2n-1}]^-'\) ions. Noting that adduct ions at \([\text{M+H}]^-'\), \([\text{M+14}]^-'\), and \([\text{M+15}]^-'\) appear in the spectra, one possible mechanism would involve the fragmentation of adducted species following ionization. This mechanism has been demonstrated in studies of fragment ions from dicyanomethane derivatives of 9-fluorenone and benzophenone.\(^3\) Alternatively, the dialkyl phthalate may undergo a gas-phase or surface-catalyzed reduction to the corresponding monoaldehyde or conversion to the monocarboxylic acid (see Scheme I), with subsequent ionization by electron-capture. Other mechanisms could involve recombination processes, as proposed by Sears et al.\(^4\)

Initially, we attempted to determine if the reaction was gas-phase or surface-assisted. Previous studies have shown that tailing ion chromatographic peak shapes, incomplete incorporation of labelled reagent gases, and a decrease in relative abundance with ion source pressure are observed for ions production involving an initial surface-assisted reaction. The spectrum of dimethyl phthalate in \( \text{CD}_4 \) showed high incorporation of deuterium. Tailing chromatographic peak shapes
were not observed. The most revealing information came from pressure studies, which showed an increase in the relative abundance of [M+H-CH4]⁺ and [M+H-OCH3]⁺ ions with ion source pressure (see Figure 3). In contrast, an increase in \( M' \) would be expected for a surface-assisted reaction. The observed behavior could be explained by an ion-molecule reaction between \( M' \) and CH4; however, experiments on a FTICR instrument showed no evidence for such a reaction. A second explanation would involve a gas-phase radical reaction. In the source, radicals are lost primarily by diffusion to the walls; recombination and flow out of the source are minor loss mechanisms. As the ion source pressure is increased, the concentration of radicals is expected to increase. Thus, the observed changes with pressure could be explained by a reaction involving gas-phase radicals.

![Scheme 1](image)

To determine if radicals were involved in formation of the [M+H-CH4]⁺ and [M+H-OCH3]⁺ ions, an emission current study was carried out. As the emission current increased, the relative abundance of the unusual ions increased. The observed change depended upon sample concentration and CH4 pressure; however, at low pressures and sample concentrations, the ratio of unusual ions/M' increased linearly with emission current. To further verify that radicals were involved in ion formation, nitric oxide was added to trap H- and CH3 radicals. Upon addition of 1% NO the abundance of the [M+H-OCH3]⁺ and [M+CH3]⁺ ions dropped dramatically. The [M+H-CH4]⁺ ion was still observed; however, its intensity also decreased with higher percentages (27%) of NO. To determine if CH3 or H radicals were involved, the spectrum of dimethyl phthalate was measured using H2 and D2 as buffer gases. While the adduct ions at [M+14]⁺ and [M+15]⁺ were not observed, the [M+H-CH3]⁺ and [M+H-OCH3]⁺ ions were still present, indicating that H radicals were involved.

Under conditions that promote formation of these unusual ions (high ion source pressure, emission current) the methane NICI mass spectra can be used to differentiate phthalates, isophthalates, and terephthalates. The terephthalates (alkyl esters of para-dicarboxylic acids) show only a molecular ion, the isophthalates (meta isomers) show the [M+H-OCH3H2n+1]⁺ ion, while the phthalates show the m/z 148 ion, [M+H-C6H2n-1]⁺, and [M+H-OCH3H2n+1]⁺ ion.

INTRODUCTION

The accurate measurement and determination of volatile organic compounds in the field requires a device which is sensitive, versatile, and easily maintained. Additionally, the device should be able to provide real time monitoring of the air being analyzed. An instrument that is not commercially available as an ambient air analysis device but does have many of the above mentioned attributes is based upon ion trap technology (1-2). A number of research groups are currently evaluating similar instrumentation for volatile organic compound analysis in ambient air (3-6). The ion trap may be operated in a variety of modes including mass-selective storage and MS/MS (7-8).

R. G. Orth and coworkers presented a paper at the 1989 EPA/APCA symposium with preliminary data obtained using the ion trap mass spectrometer (ITMS) for ambient air analysis (9). This evaluation is an extension of the initial work utilizing different operational modes and interface. The evaluation included the determination of the detection limits of the ITMS for several compounds in calibrated mixtures sampled directly. A second evaluation of air samples was performed with the ITMS.

EXPERIMENTAL

The sample introduction system consisted of a fine metering valve and a heated transfer line. An uncoated megabore (~3 meter) capillary column is used as a transfer line. The samples were introduced through a fine metering valve to control the flow rate into the device. The static pressure of sample in the device was generally 1x10^4 torr while the total pressure in the ion trap was in the 10^-5 torr range. The difference in total pressure was due to the addition of helium (added co-axially to the sample inlet stream). The samples analyzed for this analysis include a NBS aromatic standard (10 ppm and 250 ppb). Certified standards from Scott Specialty Gas of a mixture containing the aliphatic compounds (1 ppb to 10 ppm) listed in Table 1. Two air samples were collected in one liter summa vessels and were analyzed.

RESULTS AND DISCUSSION

The air standard containing chlorobenzene was admitted directly into the ITMS. The majority of the sample is N2, which makes it is difficult to detect the ppm to ppb level constituents of interest (the partial pressure of the compounds of interest is on the order of 3x10^-5 torr). To accomplish the detection at these trace levels, the device is operated in mass-selective storage mode.

The detection limits for both EI/MS and EI/MS/MS mode of operation are summarized in Table 1. The detection limits for the aromatic compounds are very conservative and are based upon data obtained from the 250 ppb and 10 ppm standards. The detection limits for the aliphatic compounds are based upon data obtained from standards ranging in concentration from 1 ppb to 10 ppm. The detection limits for all the aromatic compounds examined should be able to be improved to the low ppb to sub ppb regime. Three compounds in the mixture were unable to be quantified using the current experimental arrangement. Future work will include heating the metering valve and optimization of the data system which should allow for the quantifiable detection of all the components.

In the identification of benzene (C6H6) from one air sample, interferent compounds present make the mass spectrometric identification very difficult. The ions at m/z 77 can arise from a number of sources, but the ion at m/z 79 can arise from fewer sources such as protonated benzene or from hydrocarbons. Figure 1a shows the mass spectrum that results when the m/z ions 79 have been selectively retained. This is followed by CID of the m/z 79 ions of protonated benzene shown in Figure 1b. The fragments are typical for protonated benzene based upon knowledge of mass spectrometric fragmentation. Three components of the sample were identified by using EI/MS/MS analysis. This sample also contained ions associated with hydrocarbons which were not identified.

CONCLUSIONS

The ITMS from this evaluation has distinct promise as an analytical tool for the determination of trace organics in ambient air. The simple interface which samples air directly was shown to have detection limits in a complex background at the ppb to sub-ppb (carbon tetrachloride) levels for selected compounds. This
The limit is once again conservative and probably can be extended with improvements in the interface and data system utilized for this evaluation. The ITMS was able to identify and confirm the components of standard mixtures and samples to the ppb region using EI/MS/MS. The small size of devices based upon ion trap technology (ITD and ITS40) indicates that the device may be miniaturized to a size that would be easily transportable. Although near real time analysis was realized with this study, it should be possible with a properly designed interface and data system to obtain real time analysis.

REFERENCES


<table>
<thead>
<tr>
<th>Compound</th>
<th>EI/MS LOD's</th>
<th>EI/MS/MS LOD's</th>
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</tr>
<tr>
<td>Chloroform (84)</td>
<td>5 ppb</td>
<td>5 ppb</td>
</tr>
<tr>
<td>Toluene (91)</td>
<td>60 ppb</td>
<td>60 ppb</td>
</tr>
<tr>
<td>Chlorobenzene (112)</td>
<td>125 ppb</td>
<td>125 ppb</td>
</tr>
<tr>
<td>Carbon tetrachloride (117)</td>
<td>250 ppt</td>
<td>500 ppt</td>
</tr>
<tr>
<td>Bromobenzene (157)</td>
<td>NQ</td>
<td>NQ</td>
</tr>
</tbody>
</table>

1 - NQ - Not quantifiable with current experimental arrangement.

a) The mass-selected storage of m/z 79 which is believed to be due to the protonated benzene molecule.

b) The collision induced dissociation of m/z 79 ion which yields fragments characteristic of protonated benzene at m/z 77 and 51.
COMPARISON OF IONIZATION MODES FOR HPLC/PARTICLE BEAM/MASS SPECTROMETER INSTRUMENTATION

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Introduction

During December 1990 the Environmental Sciences Center (ESC) participated in an EPA-sponsored round robin on the analysis of benzidine and substituted benzidines using HPLC/particle beam/mass spectrometer instrumentation. That study required the electron impact analysis of blind Quality Control solutions containing benzidine, 3,3'-Cl-benzidine, 3,3'-(CH₃)-benzidine, and 3,3'-(CH₂O)₂-benzidine using ESC's recently-assembled HPLC/MS system. The same set of samples was also analyzed using methane chemical ionization. This paper compares these two modes of ionization for the quantitative analysis of this class of target compounds.

Background

New EPA methods incorporate the use of a particle beam interface for the analysis of unsubstituted and substituted benzidines.

\[ R = H \text{ for benzidine} \]
\[ = \text{Cl for } 3,3'\text{-Cl}-\text{benzidine} \]
\[ = \text{CH₃ for } 3,3'\text{-} (\text{CH₃})\text{-benzidine} \]
\[ = \text{OCH₃ for } 3,3'\text{-} (\text{CH₂O})₂\text{-benzidine} \]

This class of compounds produces large amounts of \( M^+ \) ions using 70 eV ionization and \( MH^+ \) ions using methane chemical ionization reagent ions.

\[ (\text{CH}_₃, \text{C}_2\text{H}_₅, \text{C}_₆\text{H}_{₁₅}) + X \rightarrow \text{XH}^+ + (\text{C}_₆\text{H}_{₅}, \text{C}_₆\text{H}_{₁₃}, \text{etc.}) \]

A recently-assembled system, consisting of HP-1090 HPLC with diode array detector, HP-5987 quadrupole mass spectrometer system, Vestec particle beam interface, and ESC-designed interface between the momentum separator and the EI/CI ion source, was used in this study.

Experimental

The interface between the momentum separator and EI/CI ion source consisted of a pyrex tube which inserted into the IV solids probe inlet of the ion source. Methane CI reagent gas was introduced concentric to the particle beam. Figure 1 compares the HPLC/MS chromatograms obtained under these two modes of ionization.

The following conditions were used in these analyses:

- HPLC flow rate: 1.0 mL/min.
- Gradient: 25% Acetonitrile for 1.0 min.; linear ramp to 70% Acetonitrile at 19 min.
- Injection Volume: 20 μL
- LC Column: B&J 4.6 mm x 15 cm., C₁₈, 5 micron
- No post column addition of acetonitrile was used and masses between 62 and 500 amu were scanned.

The major difference between these conditions and those of EPA's Draft Method 553 was that Method 553 suggests the use of an injection volume of 5 μL and the use of a C₁₈ LC column.
Results

The chromatograms shown in Figure 1 were obtained from the electron impact and chemical ionization analysis of a 50 μg/mL calibration standard. The Draft Method 553 requires: 1) at least a 25% valley between the dimethyl- and dimethoxy-benzidines, 2) no severe tailing, and 3) a signal/noise ratio greater than 3/1.

Calibration. External standards were used for calibration for both modes of ionization. The responses of the $M^+$ ions for electron impact, and (M+H)$^+$ ions for chemical ionization were used to construct calibration curves. Hewlett-Packard's Aquarius quantitation and identification package was used for all data reduction. Figures 2 and 3 show the calibration curves generated for this study. For all of the reported results, second order responses were assumed and the data in these two figures were used to generate a quadratic response curve upon which all blind sample concentrations are based.

Analyses. Figures 4 and 5 summarize the results of blind sample analyses using EI and CI modes. The CI data exhibit more spread than the EI data. This may be due to the lack of the use of an internal standard to correct for long term changes in instrument response.

If the % of correct value is calculated for each analyte at each concentration, the usefulness of these two modes of ionization for quantitative analysis of these target compounds can be compared. Performing this calculation yields an overall average for the % of correct value of 92.2 % for electron impact and 99.5 % for chemical ionization. The standard deviations (SDs) for these two sets of 52 data points for each mode of ionization yields a SD of 10.6 % for electron impact and 23.7 % for chemical ionization. Finally, the minimum % of correct value for electron impact was 70.4 % and the maximum value was 126.2 %. This compares with 70.2 % and 164.6 % for chemical ionization.

Conclusions

Major conclusions drawn from this study are that: 1) these four target compounds can be quantified with precision and accuracy between 10 and 30% using these two modes of ionization, 2) sensitivities are comparable using the two modes, 3) signal-to-noise is slightly higher for the chemical ionization mode, 4) second order calibration curves fit the calibration data better than first order curves, and 5) chemical ionization provides important additional information such as (M+W+1)$^+$ which may become important when analyzing unknown formulations and complex mixtures using HPLC/MS instrumentation.

Figure 3. Chemical Ionization calibration curves for benzidine and substituted benzidines.

Figure 4. Summary of blind round robin sample analyses using electron Impact ionization.

Figure 5. Summary of blind round robin sample analyses using chemical ionization.
A quantitative method for the determination of benzidines and nitrogen containing pesticides in water was developed using reversed phase liquid chromatography/particle beam/mass spectrometry (LC/PB/MS) and liquid-solid extraction (EPA Method 553; SW-846 8325). An interlaboratory study on the LC/MS portion of this method has been completed using five different types of particle beam interfaces from thirteen different laboratories. Quantitation over a 5 to 100 ng/l range for four benzidine compounds (benzidine – BZ, dimethoxybenzidine – MB, dimethylbenzidine – LB, and dichlorobenzidine – CB) was performed and the precision and accuracy for two levels (10 and 100 μg/l) are shown below.

SINGLE ANALYST PRECISION

OVER-ALL LABORATORY PRECISION

OVER-ALL LABORATORY ACCURACY
Three different extraction schemes (liquid-liquid extraction, C18 liquid-solid cartridges, and experimental neutral polystyrene divinylbenzene extraction disks - NPSDVB) were evaluated for this method. Average recovery from one liter of reagent water is shown below. Recoveries were 80 to 110% and precision for 6 to 7 replicate analyses was better than 10% for most compounds. This results in method detection limits of 2 to 25 µg/l.

**COMPARISON OF EXTRACTION SCHEMES**

<table>
<thead>
<tr>
<th>COMPOUND (ID)</th>
<th>CH2CL2 LIQUID-LIQUID EXT.</th>
<th>C18 LIQUID-SOLID CART.</th>
<th>NPSDVB EXTRACTION DISK</th>
</tr>
</thead>
<tbody>
<tr>
<td>BZ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MONURON</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CARBARYL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIURON</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIDURON</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LINURON</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROTENONE</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

On-line trace enrichment coupled with LC/PB/MS gave even lower detection limits. By extracting 100 - 500 ml of water on-line and back-flushing the entire sample onto the analytical column, 10 - 50 fold increases in method detection limits can be achieved. This will allow the analysis benzidines and nitrogen containing pesticides in particulate free water, such as drinking water, at 0.1 - 0.5 µg/l levels.

In all cases, quantitative analysis was performed using second order external calibration. The use of isotopically labeled internal standards, used in conventional GC/MS, appears to linearize the calibration curves when the ratio of analyte to coeluting labeled internal standard is plotted versus amount of analyte injected. This only appears to work with coeluting components. Thus, the use of internal standards for quantitative analysis in LC/PB/MS is limited by the need to have one internal standard for each analyte of interest. However, external calibration (as shown in the interlab study) produced acceptable accuracy and precision when sufficient quality control procedures were used. This coupled with the extraction schemes described above gives a method for the quantitative determination of benzidines and nitrogen containing pesticides in water by LC/PB/MS.
A NEW TECHNIQUE FOR THE ANALYSIS OF VOLATILE AND SEMI-VOLATILE ORGANICS

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1027 Old York Road
Ringoes, New Jersey 08551

A new accessory for the thermal desorption and direct thermal analysis of samples into a Gas Chromatograph has just been introduced by Scientific Instrument Services, Inc. This new instrument is the combined effort of Scientific Instrument Services and Rutgers University, Center for Advanced Food Technology (CAFT), who have jointly applied for patents on the Short Path Thermal Desorption System and technique.

The Short Path Thermal Desorption System (Fig. 1) permits the analysis of volatile and semi-volatile compounds by desorbing the samples directly into the GC injection port. The instrument, which attaches to the injection port of a GC, is used for the qualitative and quantitative determination of volatile and semi-volatile organic compounds present in air or compounds which can be easily purged from solid and liquid samples. The system was designed to function in two operational modes. In the thermal desorption technique, samples are sparged with carrier gas which is then passed through glass-lined stainless steel (GLT) desorption tubes packed with an adsorbent resin and/or activated carbon which traps the volatile and semi-volatile organic components. The adsorbent traps which are fitted with a syringe needle, are subsequently thermally desorbed directly into the GC injection port for separation and determination. In an alternate technique termed direct thermal analysis, solid samples of low moisture content are placed directly into the GLT desorption tube where they can be heated and purged to direct the outgassed organic compounds directly into the GC. This permits the samples thereon to be injected, heated, and desorbed directly into the injection port via the shortest path possible.

The Short Path Thermal Desorption System consists of an Electronics Control Unit and a Desorption Unit which mounts directly on top of the GC injection port. The unit is easily movable and transferable since no mounting bolts or hardware are needed for installation. Due to its novel, inert short path of sample flow, this new system overcomes the disadvantages of other desorption systems by eliminating long transfer lines, thereby providing for the optimum delivery of sample to the GC resulting in increased sensitivity. Labile samples are not lost or destroyed in hot transfer lines and no "memory effects" occur due to contamination of transfer lines since each sample analyzed constitutes a new independent pneumatic circuit. The technique is fully compatible with capillary and packed column operation and may be used with all types of GC detectors including systems interfaced to mass spectrometers or Fourier transform infrared spectrometers. Parameters such as temperature and desorption time are accurately controlled.
controlled via a digital electronic control module. Samples can be ballistically heated throughout the temperature range of ambient to 350°C. Features such as auto-injection of sample and carrier gas flow control are accomplished using precision pneumatic circuitry.

Samples such as volatile and semi-volatile organics in air, flavors and fragrances in foods and cosmetics, manufacturing chemical residues in pharmaceuticals, volatiles in packaging materials and building products, and aromatic residues in Forensic arson samples are just a few of the applications for which this technique has been utilized. Additionally the technique has been applied for specific applications such as the detection of benzene and chlorinated hydrocarbons in food and other manufactured products, the identification of natural occurring insect attractants in plants, the identification of flavors in black pepper and other spices, and the identification of volatile contaminants in commercial shipping containers. The technique eliminates the need for solvent extractions in many analyses.
Integration of Methods for Measuring Human and Wildlife Exposures to Pollutants

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In spite of extensive characterization of environmental pollution, our knowledge of the levels of exposure of humans and wildlife to pollution has lagged and we know even less about the health effects of pollution. One important observation complicates this situation: the toxicity of complex mixtures is poorly understood yet it is precisely these complex mixtures that are typically discharged into the air, water, and soil. Furthermore, what constitutes toxicity is not often well defined. An interdisciplinary approach is called for which integrates the data from chemical analyses with various measures of biological endpoints. We have found it useful to have an array of mass spectrometric techniques at our disposal in addressing these issues.

Urinary Mercapturic Acid Indicators of Exposure: GC/electron capture MS

The appearance of mercapturic acid conjugates in urine may serve as a useful indicator of exposure to numerous xenobiotic chemicals. Conventional GC/electron capture MS of esterified urine extracts has found limited success owing to the extensive fragmentation of these compounds. In contrast, we have found that GC/electron capture ionization of pentafluorobenzyl esters of mercapturic acid conjugates yields sensitive (~1 ppb) detection of a variety of conjugates in urine with minimal sample clean up. Our experiments have demonstrated the formation of several isomeric conjugates in the urine of model animals exposed to 4-vinylcyclohexene. The mercapturic acid conjugate of 4-hydroxy-2-nonenal is excreted in the urine of rats exposed to CCl4. This reactive aldehyde is a product of peroxidative damage of membrane lipids that results from oxidative stress.

Synthesis of α,ω-mercapturic acid standards is straightforward, and the use of these stable isotope labelled internal standards allows for easy quantification. The electron capture spectra of all mercapturic acid conjugates studied to date yield a peak at m/z 128 which is often the base peak. This peak arises from the N-acetylcysteine moiety, and allows these conjugates to be more readily detected in urine extracts. Recent experiments have demonstrated that mercapturic acid derivatives not amenable to GC separation can be detected using negative continuous-flow FAB ionization.

Incineration of Chlorinated Solvents: GC/MS and Laser Desorption MS

As part of a study of the incineration chemistry of chlorinated solvents such as trichloroethylene, we designed and built a laser desorption time-of-flight mass spectrometer for the direct analysis of soot. The instrument is capable of single step desorption and ionization using either Nd:YAG (λ = 266 nm) or CO2 (λ = 10.6 μm)
lasers. The 266 nm harmonic can also be Raman-shifted to yield 239 or 299 nm radiation to vary the selectivity of ionization.

Single step ultraviolet (266 nm) laser desorption/ionization of compounds adsorbed onto soot particles allows for a rapid screening of soot for polycyclic aromatic hydrocarbons (PAHs) because of the enhanced efficiency of 2-photon resonant ionization relative to non-resonant photoionization of aliphatic and monoaromatic constituents. Some photodissociation has been observed in these single shot experiments, but this is markedly reduced when infrared desorption is used followed by ultraviolet photoionization of the desorbed neutrals.

The ability to screen soot samples for PAHs allows us to select combustion experiments for which the combustion gases are trapped on sorbent tubes (Carbotrap CO). Splits of the extracts are characterized using GC/MS, and aliquots are distributed to collaborators who measure biological endpoints using cell culture and lung airway explant techniques.

Petroleum Marker Compounds Discharged from Oil Recovery Operations

An important issue surrounding coastal oil drilling is the discharge of produced water from these activities and the potential risk to marine wildlife. It is particularly important to distinguish the inputs which arise from oil recovery activities from seeps which occur naturally in coastal waters.

Routine processing of GC/MS data typically yields a complex array of mostly indistinct hydrocarbons. However, petroleum geochemists have used extracted ion current profiles of characteristic biomarker compounds to characterize petroleum source rock. In conjunction with a group studying the population ecology of marine wildlife, we have applied this organic geochemical methodology to the characterization of crude oil produced water and marine sediments taken from the Santa Barbara channel off the coast of California. Our initial results suggest that the relative amounts of saturated, monoaromatic, and triaromatic steranes are indicators of biodegradation and can be used to assess the efficacy of secondary treatment of produced water as well as to distinguish weathered inputs from natural seeps from recent discharges of produced water. We are currently performing analyses of mussels collected in the vicinity of a produced water outfall to determine whether this methodology can be used to measure the exposure of marine organisms to produced water constituents.
We have determined operating conditions for ion trap GC/MS systems which minimize the common problems associated with unstable quantitative precision and inaccuracy in the analysis of volatile organic chemicals (VOC) in water. Our research has illustrated the important contribution of ion trap helium pressure and water/methanol background concentrations common with purge-and-trap sample introductions. Concurrent developments with capillary columns (J&W Scientific), liquid sample concentrators (Tekmar Company), and capillary jet separation interfaces (SGE) have in combination significantly advanced the use of ion trap mass spectrometry for ultra-trace VOAs.

A specially designed all glass-lined stainless steel capillary jet separator interface has been developed to maintain the helium pressure required for an ion trap mass spectrometer. Figure 1 shows the proper working range of input and output flow rates for this interface with actual data from our three development systems. By use of interchangeable collector jets, both low flow (5-10 ml/min) and high flow (10-20 ml/min) chromatographic systems are accommodated. At the optimum of 1.3 ml/min output flow rate, the jet separator efficiency has been measured to be 43-54% for all USEPA regulated VOCs. The common matrix background of aqueous samples and analytical standards has been studied using an ITMS to elucidate the possible ion-molecule reactions affecting response factor stability and detection limit. Under conditions of high flow (18 ml/min) using a megabore (0.53mm) 75M VOA column, the co-elution and resultant ion-molecule reaction products of exogenous water and methanol are illustrated in Figure 2. An example of the deleterious effects caused by excessive water and methanol levels is shown in Figure 3 for chloroethane. By operating the extended length megabore VOA column at high flow rates, sufficient backpressure can be achieved to lower the partial pressure of water and methanol during desorption of the chemical trap. In series with the transfer line of the purge-and-trap, a novel thermo-electric condensation trap has been implemented to further reduce the water and methanol levels entering the chromatographic system. By the combination of these techniques, our measurements have shown a 60-75% reduction of this background. At this level, the ion trap mass analyzer can then be set for an effective RF cut-off at m/z 32 to exclude the residual amounts.

It is by the combination of partial pressure suppression, condensation and mass exclusion, that now yield a reproducible operating condition for the analysis of VOCs at sub-ppb levels. Figure 4 illustrates the improved statistical precision based on response factors for the early eluting VOCs (0.5 to 20 ug/L). While calibrating as high as 20 ug/L, our studies have shown MDLs at or below 0.05 ug/L for the USEPA 524.2 analytes. Figure 5 illustrates the high resolution chromatographic results achievable using the capillary jet separator as an interface. By use of these advances, it is currently possible to operate the ion trap mass spectrometer for higher sensitivity, below the quantitation range of USEPA method 524.2 to levels approaching 0.001 ug/L.
ITMS™ Experiments Uncover Fundamental Reaction Principles

As an example, the common quantitative instability of the purgeable gases with all mass spectrometers, can in part, be explained by the understanding of ion molecule chemistry as shown here for Chloroethane reacting with high levels of exogenous water and methanol.

\[
\begin{align*}
\text{CH}_2\text{CHCl}^+ + \text{H}_2\text{O} & \rightarrow \text{CH}_2\text{CH}_2\text{Cl} + \text{H}_2\text{O}^+ \\
\text{m/z} & 64 \\
\text{CH}_2\text{CHCl}^+ & \rightarrow \text{CH}_2 + \text{CH}_2\text{Cl}^+ \\
\text{m/z} & 64 \\
\text{CH}_2\text{Cl}^+ + \text{CH}_3\text{OH} & \rightarrow \\
\text{CH}_2\text{Cl}^-\text{CH}_2\text{OH} & \rightarrow \text{CH}_3\text{CH}_2\text{O}^+ + \text{HCL} \\
\text{m/z} & 45
\end{align*}
\]

Figure 3.

Improved Response Factor Precision for VOC "Purgeable Gases"

The best statistical precision for the early eluting gases results from the combined use of extended length VOA capillary columns with sub-ambient initial temperature, high flow capillary jet separation interfacing, and the water background reduction provided by the Tekmar 2000 LSC with Moisture Control Module.

Figure 4.

Total Ion Chromatogram for a 5.0 ug/L 524.2 VOC Standard

Figure 5.
Atmospheric pressure chemical ionization mass spectrometry makes possible the detection of several atmospheric species at concentrations down to or even below the 1 part per quadrillion level. The number of species for which such measurements are possible is, however, small. The only species measurable with this sensitivity are those which either have very high proton affinities (or low ionization potentials) or those which are very acidic (or have high electron affinities) in the positive and negative spectra respectively. Sulfuric acid is an example of one of these species for which such sensitive measurements are possible. Figure 1 shows a recent measurement of the atmospheric gas phase concentration of H$_2$SO$_4$ as a function of time of day during April 20, 1991 made at Cheeka Peak Research Station near Neah Bay, Washington. If somewhat less alkaline or acidic species are to be measured, they must normally be present at higher concentrations. These species may then be measured by insuring that the ion/neutral reaction time is short enough so that the ions of interest will not be significantly removed by higher proton affinity or more acidic species in the positive and negative spectra respectively. Even when this condition can be insured using normal chemical ionization methods, there still remains a great deal of uncertainty surrounding the initial ionization process. Typically, chemical ionization of the species of interest is accomplished through reactions with several often unknown initial ion species and involves a multiplicity of unknown exothermicities and reaction rate constants. Thus, the number of ions formed from a trace species of interest is in general not a well known or characterized function of its concentration. Instead the ions of interest may depend in part on the number and concentration of other trace species present. If highly exothermic reactions are involved in the chemical ionization of the species of interest, the reaction may result in the formation of daughter or fragment ions making the measurement even more difficult. In order to simplify the reaction schemes leading to the ionization of a product ion of interest, dilute mixtures of reactive gases such as benzene have been added to the chemical ionization buffer gas. As a result, a single known initial reactant ion can be formed (such as C$_6$H$_8$•*), thus considerably reducing the number of reactions leading to the product ion of interest.

Several uncertainties, however, remain: 1) do the radical or metastable species formed in the ionization process itself interfere with the measurement, 2) does the relatively large reactive buffer gas component sometimes used to control the initial reactant ion identity have secondary effects on either the ion or neutral chemistry, and 3) how does one prevent the removal of product ions of interest by the formation of still more stable ion species?

The present concept of controlled high pressure selected ion chemical ionization mass spectrometry (HPSICI/MS) promises to remove the above uncertainties and the associated measurement problems for a wide variety of difficult-to-measure species. This is accomplished using a variety of techniques (many of which are quite new) to independently control the ion formation process, neutral species preparation, the ion/neutral reaction environment, and ion sampling and analysis techniques. Production of a single initial ion species is accomplished by the addition of reactive buffer gas additives in an isolated ionization region of the measurement apparatus. Thus interferences from metastable and radical species as well as those from buffer gas additives (if they pose a problem), are removed from the chemical ionization region. The preparation of neutral species of interest can be accomplished by: titrating the species into the parent species of a more stable ion (ex. titration of the atmospheric hydroxyl radical into sulfuric acid$^6$, gas chromatographic separation (ex. GC separation of dimethylsulfide, and beta-caryophyllene)$^7$, or any other appropriate separation or enhancement technique. Control of the ion/neutral reaction environment includes variation of temperature pressure and the addition of reactive species that assist in forming more stable product ions (ex. SO$_2$ measurement using the SO$_2$•- product ion)$^8$. Finally, the sampling and analysis of reactant and product ions may involve collisionally inducted dissociation prior to analysis$^9$, followed by MS or MS/MS analysis.
As a result of these chemical ionization control techniques, GC/HPSICI/MS has been used at concentrations down to and even slightly below 1 pptv (part per trillion). Titration assisted HPSICI/MS has been successfully used down to the low ppq (part per quadrillion) concentration range, and direct HPSICI/MS has proven to be applicable down to and even below the 1 ppqv concentration range.

This work was supported by the National Science Foundation under grant Nos. ATM 8717675 and ATM 9021522, the Office of Exploratory Research/United States Environmental Protection Agency under contract No. R-817121-01-0 and under internal Georgia Tech Research Institute grants, Nos. E 904-023 and E 8904-046

References

Figure 1. Gas phase sulfuric acid concentration in molecules/cm$^3$ as a function of time of day measured on April 20, 1991. The large diurnal variation in sulfuric acid concentration results because its formation is dependent on a photolysis product (OH).
MEASUREMENT OF ELECTRON CONCENTRATIONS IN THE LOWER ATMOSPHERE BY CHEMICAL RELEASE MASS SPECTROMETRY

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Phillips Laboratory/LID, Hanscom AFB MA 01731-5000

Introduction
The measurement of electron density throughout the mesosphere has proven to be a challenging problem. Both in-situ measurements and remote sensing techniques have been plagued by the very low electron densities involved (10^{-2} to 10^{4} \text{ cm}^{-3}). In addition, in-situ techniques are beset with the effects of the fast motion of rocket probes through regions of varying pressure (0.01 to 1 Torr), which results in transitional flow past the probes. This in turn is very difficult to model.

We have developed a new measurement technique which has alleviated some of the typical electron measurement problems. The technique involves releasing SF_6 in front of a negative ion mass spectrometer. Electrons are converted to SF_6^- and SF_5^-, and the ratio of electrons to negative ions is measured by measuring the ratio of the SF_6^- plus SF_5^- ion signals to the sum of all other negative ion signals. When combined with modelling of the neutral flowfields surrounding the rocket probe, the technique shows promise of being able to measure electron densities down to 0.04 electrons cm^{-3}.

Technique
The technique involves releasing a controlled amount of SF_6 in front of a rocket borne mass spectrometer. SF_6 is known to attach electrons rapidly to form SF_6^- and SF_5^- with a rate constant on the order of 10^{-7} \text{ cm}^{3}\text{ s}^{-1}. The branching ratio between these two channels is known to depend on the temperature and the kinetic energy of electrons but the rate constant is independent of temperature. Other ions in the atmospheric altitude range of interest (40-90 km) will not react with SF_6 under the conditions of the release, nor will SF_6^- or SF_5^- react appreciably with the ambient neutrals. Table 1 shows the relevant chemistry and the time scales of the various processes. The time scale for electron attachment is extremely short, orders of magnitude shorter than that for any ion chemistry that might take place. This enables an SF_6 release rate to be chosen such that all electrons are converted to SF_6^- and SF_5^- without any interfering ion chemistry occurring. The fraction of negative charge in the form of electrons is then just the ratio of the sum of the SF_6^- and SF_5^- ion signals to the total ion signal. The electron concentration is put on an absolute basis by separately measuring the total positive ion concentration realizing that the total positive and negative charge concentrations are equal.

The flight conditions were chosen as follows. The injector effuses SF_6 at a rate of 6 \times 10^{-3} \text{ standard cm}^{3}\text{ s}^{-1} in order to maintain an SF_6 gas concentration of 1 \times 10^{11} \text{ cm}^{-3}. The spacing between the injector and the entrance to the mass spectrometer was chosen to allow at least five electron-SF_6 attachment time constants to ensure complete electron attachment. Neutral gas and ions are drawn into the mass spectrometer, where the ions are separated according to their mass to charge ratio and counted with a channel electron multiplier. The interior of the mass spectrometer is maintained at high vacuum with a liquid helium cooled cryopump. The
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mass spectrometer was capable of measuring molecular species of up to 175 amu and had mass resolution of 75. The SF₆ release was started on the downleg part of the rocket flight at an altitude of 85 km. A experimental problem occurred shortly thereafter, and only data at 85 km was obtained.

Table 1. Chemistry Relevant to Electron Detection by a Controlled SF₆ Release.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate constant (cm³ s⁻¹)</th>
<th>Reaction Time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>e⁺ + SF₆ → SF₆⁻ (99.5%)</td>
<td>3.7 × 10⁻⁷</td>
<td>1.4 × 10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>→ SF₆⁻ + F (0.5%)</td>
<td></td>
</tr>
<tr>
<td>O₂⁻ + SF₆ → SF₆⁻ + O₂</td>
<td>3.3 × 10⁻¹¹</td>
<td>1.3 × 10⁻³</td>
</tr>
<tr>
<td>O⁻ + SF₆ → SF₆⁻ + O</td>
<td>endothermic</td>
<td></td>
</tr>
<tr>
<td>Cl⁻ + SF₆ → products</td>
<td>endothermic</td>
<td></td>
</tr>
<tr>
<td>NO₃⁻ + SF₆ → products</td>
<td>endothermic</td>
<td></td>
</tr>
<tr>
<td>HSO₄⁻ + SF₆ → products</td>
<td>endothermic</td>
<td></td>
</tr>
<tr>
<td>SF₆⁻ + O₂ → products</td>
<td>endothermic</td>
<td></td>
</tr>
<tr>
<td>SF₆⁻ + O → products</td>
<td>endothermic</td>
<td></td>
</tr>
<tr>
<td>SF₆⁻ + O₂(¹Δ) → products</td>
<td>≥ 5 × 10⁻²</td>
<td></td>
</tr>
</tbody>
</table>

Results

The negative ion composition at 85 km before and after the SF₆ release is shown in Table 2. The effect of the SF₆ release is evidenced by the two percentages at mass 146. Before the release no atmospheric source of mass 146 was detected; after the release nearly 17% of the negative ion signal came from SF₆. Comparing the ratio of mass 146 ions to the sum of all other ions and estimating the unperturbed ambient ion density at 3000 ions cm⁻³ yields an estimated 500 electrons cm⁻³. This compares favorably with modelled electron densities for quiet mid-latitude daytime conditions. With the count rate observed, the instrument has a detection limit of 0.04 electrons cm⁻³ in accord with the preflight estimate. Therefore although the instrument failed shortly after the SF₆ release began, the feasibility of the technique has been demonstrated.

Table 2. % of Major Ionic Products Before and After the SF₆ Release at 85 km.

<table>
<thead>
<tr>
<th>Mass</th>
<th>% Before SF₆ release</th>
<th>% After SF₆ release</th>
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<tbody>
<tr>
<td>16</td>
<td>4</td>
<td>3</td>
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<tr>
<td>80</td>
<td>0.4</td>
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<tr>
<td>97</td>
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<td>17</td>
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<tr>
<td>160</td>
<td>87</td>
<td>73</td>
</tr>
</tbody>
</table>

1294
Mass Spectrometric Measurements of the Space Shuttle Environment

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Hanscom AFB, MA 01731

Mass spectrometry is a valuable diagnostic for determining the identities and concentrations of gaseous contaminants in the Space Shuttle environment (1). These contaminants arise from surface outgassing, payload venting, cabin leakage, thruster firings, and other spacecraft system operations. Water vapor is one of the most important gaseous contaminant species. Because water adheres strongly to surfaces, the time decay of water concentration in the shuttle environment is slower than for many other gases, and its concentration is larger.

We have measured water vapor concentrations in the payload bay (along with many other neutral and positively charged species) on two shuttle flights. The first time was on the fourth flight of the Shuttle (STS-4) in the summer of 1982, and the second was on the recently-completed STS-39 flight of Discovery from April 28 to May 6, 1991. These two data sets provide an opportunity to compare water concentrations and time dependencies under the differing circumstances of the two flights.

The instruments on both flights were essentially identical, compact quadrupole spectrometers. They were mounted in a fixed position in the payload bay with a viewing direction perpendicular to the long axis of the shuttle and slightly above horizontal over the right wing.

The water vapor data from STS-4, which have been discussed previously (2), are shown in Fig. 1. The water signal (in instrumental units) is plotted in the top panel and the instrument temperature is plotted below. The strong correlation between temperature and water concentration is due to an increased surface outgassing rate at higher temperatures. Although not as clear from the figure, the absolute amounts of water (i.e. concentration at one temperature) also decreases somewhat as a function of time.

Analogous data from the recent STS-39 flight are shown in Fig. 2. These very preliminary data were recorded by hand during the flight, and should only be used to indicate broad trends in water concentration. The water concentration is plotted in raw telemetry volts, which is a logarithmic scale where each volt change represents a factor of ten in actual concentration. The two temperatures plotted in the lower panel are for the mass spectrometer sensor (under thermal blankets) and the payload structure near the instrument (exposed).

Many of the same trends appear that were observed in 1982. Increases in temperature again lead to increases in water concentration, as seen at Mission Elapsed Time (MET) 80-100. The overall decrease in water signal as a function of time through the flight is clearer than in the earlier data.

Two different instrumental units for the water signal were intentionally used to avoid direct comparisons between the two data sets until after post-flight calibrations of the STS-39 instrument. However, a preliminary look at the pre-flight calibrations suggests that the STS-39 water concentrations were approximately as large as during STS-4. This is surprising, given that the tiles on Columbia were not treated with water repellant before STS-4 and the orbiter was rained on just before launch. STS-39 should have been a much drier flight.
A possible explanation lies in the fact that STS-39 flew at a lower altitude than STS-4 (260 km vs 305 km), and that May 1991 is closer to solar maximum than July 1982 was. For both of these reasons, the atmospheric density during STS-39 may have been significantly higher at the orbiter altitude than during the earlier flight. As the orbiter moves through the atmosphere at high velocity, a "plow cloud" of gas forms on the ram side of the vehicle which may be a factor of ten more dense than the ambient atmosphere. Even if the outgassing rate from the STS-39 surfaces was lower than STS-4, that outgassing flux could have been collisionally "trapped" within a higher density plow cloud, leading to longer residence times of water molecules near the shuttle and a correspondingly higher pressure.

References


The Giotto spacecraft flew by comet Halley on the night of 13/14 March 1986 at a closest approach distance of about 600 km. One of the instruments on board the spinning spacecraft was the Ion Mass Spectrometer (IMS), designed to measure the composition and three-dimensional velocity distributions of ions in the Halley coma. This paper discusses the design of the IMS and some of the results obtained at the comet.

The wide range of plasma conditions anticipated, as well as the very short time available for measurements (because of the 68 km/s flyby speed) required some unique design solutions for this mass spectrometer. The instrument consists of two sensors. The High Energy Range Spectrometer (HERS) was optimized for measuring the solar wind interaction with the comet and the hot, outer portion of the coma. At the entrance to the sensor the ions are first reflected by an electrostatic mirror which compresses the 60 degree external field of view into 30 degrees internal to the sensor. The mirror also helps keep the dust particles from entering the instrument. A pair of grids then pre-accelerate or decelerate the ions to the velocity necessary to pass through the 120° sector magnet constant momentum filter. The magnet has symmetrically placed entrance and exit slits, which results in unity magnification. The magnet exit slit is also the entrance slit of an electrostatic deflector with a field that is non-uniform in a plane normal to the entrance slit. Since all ions enter this deflector with a single momentum set by the magnet, different mass/charge results in different velocity (and thus energy). Mass/charge is therefore separated along the deflector. The symmetry of the magnetic momentum filter results in angles being preserved from entrance to exit slits. Thus mass/charge is imaged along one direction of the deflector exit plane and incident angle along the perpendicular direction. A two-dimensional microchannel plate detects the ions at this exit. The instrument design allowed mass/charge ranges 2-4 amu/e, 12-26 amu/e, 16-34 amu/e and protons only to be measured over an energy range from 10 eV/e to a mass dependent upper limit of ~4keV/e.

The second sensor, the High Intensity Spectrometer (HIS) was optimized for the denser and cooler plasma of the inner coma. In this sensor, two independent detector systems allow, separately, high angular resolution in the relative velocity direction, and good mass resolution in the range 12-56 amu/e. The energy and angles are measured with an electrostatic quadrupole analyzer, while the mass analyzer uses a permanent magnet between two quadrupole lenses to image ions onto a linear array of channeltrons. The design gave a mass/charge range of 12 - 56 amu/e from 300 eV/e to 1500 eV/e in energy.

The instrument performed well during the comet encounter. Analysis of the returned data gave some expected as well as unexpected results. As anticipated, most of the coma is dominated by ions resulting from the dissociation, ionization, and subsequent ion-molecule reactions of the chief constituent water. The surprises included the higher than anticipated amount of C+ and the lower than expected level of nitrogen. The plasma dynamics observed was also quite interesting: the weak bow shock observed (at around 1 million km from the nucleus) apparently resulted more from the mass loading of the solar wind by the newly born picked up ions than from the presence of the nucleus as an obstacle. These pickup ions were also scattered in phase space by a host of plasma waves, more so than theory had previously predicted. At about 4000 km from the nucleus a sharp boundary was found (sometimes called the ionopause or contact surface) which separated, on the inside, the relatively cold (~300K) nucleus-dominated and almost stagnant ion population from the hotter, flowing comet-solar wind mixture on the outside. A population of fast ions was observed streaming inward near this boundary, postulated to result from charge exchange of pickup ions to neutrals in the outer coma and then again back to ions as the neutrals enter the dense region of the inner coma.

(Supported in part by a contract with the US National Aeronautics and Space Administration.)

Interstellar gas clouds and circumstellar envelopes are the largest known chemical factories in the universe. A part of the chemistry in these environments is driven by ionizing radiation which initiates ion chemistry directed toward the growth of ions and molecules in the gas phase. Infrared and radio-astronomers have observed many different ions and molecules in these environments and chemical models have been proposed and developed by astrophysicists in order to account for their abundance. The laboratory ion chemist obtains information about the kinetics and product distributions for individual ion/molecule reactions and these provide the basis for the identification of the plausible chemical networks which are responsible for the growth of ions and molecules by ion chemistry.

The newest molecules which have been identified in circumstellar and interstellar environments are largely carbonaceous, highly or completely unsaturated and chain-like, and, it should be noted, all are carbenes. They include dicarbenes such as :CCCCC:, radical carbenes such as :CCCCCCCCH:, carbenes with two H-atoms at one end such as :CCCH₂ and :CCCHH₂, and carbenes with one terminal heteroatom such as :CCCO, :CCCS and :CCCCSi. Also, there now is direct evidence for the cyclic carbenes (silenes) c:-C₃H₃, c:-C₂H, and c:-SiC₄, and indirect evidence for sheets of carbon atoms in the form of free or bound PAH molecules and as grains of graphite, and hollow cages of carbon atoms from the fullerene family of molecules, such as C₆₀, also have been proposed to be present. Many of the observed and postulated carbon species occur in similar circumstellar or interstellar regions and so are expected to share a common chemistry.

Of course both neutral and ion chemistry may contribute to the gas-phase growth of molecules in an ionized medium and they do so in a cooperative fashion with ion chemistry becoming relati-
very more important at lower temperature and higher levels of ionization. Ions produced by ion-
molecule reactions need to be neutralized to produce molecules and the desired bonding must be pre-
served in the neutralization step. Such neutralization may occur, for example, by charge transfer and
proton transfer or recombination with electrons. Laboratory investigations and other considerations in-
dicate that the formation of interstellar and circumstellar carbonaceous molecules by ion chemistry may
occur in the following manner:

\[ \text{:C}_n \text{ from C}_n \text{H}^+ \] and \[ \text{:C}_n \text{H from C}_n \text{H}_2^+ \]: Association of carbon ions with carbon-chain mole-
cules and the subsequent hydrogenation of the carbonaceous adduct ions in sequential reactions with
\( \text{H}_2 \) (which stop at \( \text{C}_n \text{H}_2^+ \)) or by chain-lengthening reactions with \( \text{C}_n \text{H}_2, \text{C}_n \text{H}_4 \) and \( \text{C}_n \text{H}_6^+ \).

\[ \text{:C}_n \text{H}_2 \text{ from C}_n \text{H}_2^+ : \] \( \text{H}_2 \) adds to \( \text{C}_n \text{H}^+ \) to give \( \text{I-C}_n \text{H}_2^+ \). \( \text{C}_n \text{H}^+ \) reacts with \( \text{CH}_4 \) to produce
\( \text{C}_n \text{H}_2^+ \), and \( \text{CH}_2^+ \) reacts with \( \text{C}_n \text{H} \) to produce \( \text{C}_n \text{H}_3^+ \). Also possible are homologous series of reactions of
the type \( \text{C}_n \text{H}_n^+ + \text{CH}_4 \) and \( \text{CH}_2^+ + \text{C}_n \text{H}_6 \) leading to even and odd carbon chains of the type \( \text{:C}_n \text{H}^+ \).

\[ \text{:C}_n \text{X from XC}_n \text{H}^+ \text{ where X=O,S and S}: \] By the transfer of an X-atom from a molecule XY
to \( \text{:C}_n \text{H}^+ \) or simply by the addition of \( \text{:CX} \) molecules to \( \text{:C}_n \text{H}^+ \), by C-H insertion of an atomic ion X- into
\( \text{C}_n \text{H}_2 \) or by the C-H insertion of the atom X or molecule \( \text{:CX} \) into \( \text{C}_n \text{H}_2^+ \).

\( \text{c-}\text{:C}_n \text{H}_2, \text{c-}\text{:C}_n \text{H and c-}\text{:SiC}_2 \) from their protonated cyclic (or linear) forms: \( \text{c-}\text{C}_n \text{H}_2^+ \) can
be formed from \( \text{C}_n \text{H}^+ + \text{CH}_4 \) or \( \text{CH}_2^+ + \text{C}_n \text{H}_2 \) but the result of its recombination with electrons is uncer-
tain. The situation is similar for \( \text{c-}\text{:C}_n \) and \( \text{c-}\text{SiC}_2 \). Other possibilities exist for the formation of
\( \text{c-}\text{:C}_n \text{R}_1 \text{R}_2 \) where \( \text{R}_1=\text{H}, \text{CN}, \text{CH}_2 \text{CN} \) and \( \text{C}_n \text{CN} \).

**Benzene, naphthalene, PAH and sheets of carbon:** Reactions of \( \text{CH}_2 \text{CCH} \) and
\( \text{H}_2 \text{CCCH}_2 \) with their ions produce protonated benzene. Ionized benzene reacting with \( \text{C}_n \text{H}_2 \) produces
ionized naphthalene. Further reactions with \( \text{C}_n \) or \( \text{C}_2 \) units may form larger PAH molecules and possibly
graphite, the end member of the PAH series.

**Saucers and cages of carbon:** "Clamping" with C or \( \text{C}_2 \) units in sequential PAH-forming
reactions could introduce curvature to produce carbon saucers and ultimately the cage-like molecules
known as fullerenes.
Quantitative Aspects of LC/Particle Beam/MS

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M. L. Vestal, Vestec Corporation, 9299 Kirby Drive, Houston, Texas 77054

The task of mating High Performance Liquid Chromatography (HPLC) with Electron Impact Mass Spectrometry (EI/MS) has been accomplished by means of designs which first produce an aerosol from the liquid mobile phase and then dry the aerosol droplets to their non-volatile residues. The resulting particles are carried into a jet separator in which the large momentum particles are segregated from the low momentum molecular species and are injected as a beam into a Gas Chromatography EI/MS ion source. Spectra obtained are library searchable.

Quantitation is possible with LC/MS data but suffers from: non-linearity of response; "Carrier effects" which inappropriately enhance response for co-eluting substances (such as deuterated internal standards); day-to-day instability of response which necessitates either the use of internal standards or frequent recalibration; and huge variations in sensitivity depending on the mobile phase flow rate.

The work reported here was divided into two parts. Part I dealt with the effort to use an internal standard in a particular project and to define the conditions under which it would (or would not) improve the accuracy of determinations. The conclusions were that the compound chosen, though very dissimilar to the target compounds of the analysis, served as an excellent internal standard for a target compound which eluted in the same portion of the LC gradient, but was no help at all for correcting the determination of a compound which eluted in a different solvent mixture. First and second order calibration curves were also constructed and their usefulness compared.

Part II involved modification of several of the elements of the system to improve Limits of Detection, linearity, and chromatographic fidelity. Significant changes were made to the vaporizer/nebulizer, the spray chamber, the jet separator, and the ion source. Quantitative figures of merit were measured and presented in tabular form to demonstrate the effects of each design change on the system performance. The design changes made great differences in the innate sensitivity of the system for medium and large concentration components but did not materially improve detection limits for the small particles produced from trace components. Addition of a semi-volatile salt, ammonium acetate, to the LC mobile phase dramatically lowered detection limits and permitted the new designs to demonstrate excellent figures of merit. This is an example of the positive use of "carrier effects" because the ammonium acetate was removed with much of the solvent vapors in a diffusion cell prior to the jet separator. Thus very little of it was transmitted through the jet separator and into the mass spectrometer; the spectra were not adversely affected. The fact that a semi-volatile buffer can so dramatically increase transmission of smaller particles to the source implies that the major place where they are lost is not by discrimination in the
jet separator but earlier, probably at the time when the aerosol transport system is first being set up.

The new ion source has combination EI and CI capability. A simplified diagram of this source is shown below.

![Particle Beam/Mass Spectrometry Ion Source](image)

This work demonstrated the practicality of particle beam sample introduction into the relatively high pressure region of a CI source. For benzidine the CI detection limit was found to be about 200 pg/s compared to a best case EI detection limit of less than 100 pg/s. Without the ammonium acetate buffer typical detection limits were over 2000 pg/s.
Chemical Ionisation combined with Particle Beam LCMS for magnetic sector mass spectrometers.

M.R. Green, R.H. Bateman, C. Lewis and P.A. Bott

Fisons Plc., VG Analytical, Floats Road, Wythenshawe, Manchester, M23 9LE, England.

Particle Beam LCMS has proven to be a useful technique for a wide range of compounds of medium to low volatility and polarity. Many compounds may be ionised directly by electron impact giving readily interpretable or library searchable spectra for nanogram quantitation levels. However, the ability to ionise the particle beam by chemical ionisation opens up the possibility of studying compounds which give little molecular weight information by El.

In the El mode of operation the particle beam is transported from the final skimmer to the ion source without the need for any guidance of the beam. Any nebulising gas or solvent remaining after the two-stage momentum separator is pumped away. For CI operation a gas-tight source is required to give good chemical ionisation conditions at reasonable reagent gas flow rates. Therefore, a sealing arrangement is required between the particle beam interface and the source. Due to the relatively high gas pressures resulting in high gas flows, both from the particle beam and the CI reagent gas, electrical discharges may occur on magnetic sector mass spectrometers with accelerating voltages of several kilovolts.

A tube was constructed to form a transfer line from the exit of the second skimmer to the source. The internal diameter of this is 2mm which allows all of the particle beam to enter the source without attenuation. To maintain molecular flow and prevent electrical discharge two pumping holes were added to the tube near to the momentum separator, but pumped by the source diffusion pump. This arrangement allowed the mass spectrometer to be operated at 8000V accelerating voltage with a good CI plasma, and the particle beam interface in operation.

Data was obtained for several compounds to test the quality of the CI arrangement. Figure 1 shows the El spectrum and library spectrum for the insecticide carbofuran, using 50ng of sample. The molecular ion peak at 221 Da is typically only 6% of the base peak at 164Da. This compound readily loses CH3NHCO to yield the benzofuranol ion at 164Da. Figure 2 is the CI mass spectrum obtained from 50ng of carbofuran using methane as reagent gas. The spectrum clearly shows the pseudomolecular ion (222 Da) together with the C2H5 adduct at 250Da. Also the benzofuranol ion is protonated (165 Da) and its C2H5 adduct is observed at 193Da.

Good quality CI data can readily be obtained under CI conditions using a particle beam interface or magnetic sector mass spectrometers utilising the full instrument sensitivity at full accelerating voltage.
Figure 1 50ng CARBOFURAN FULL SCAN EI

Figure 2 50ng CARBOFURAN, METHANE CI
THE DEVELOPMENT OF AN ADVANCED, HIGH SENSITIVITY BENCHTOP LC/GC/MS SYSTEM

D. S. RICHARDS
VG MASSLAB LTD

Until now, particle beam LC/GC/MS systems have been large, floor standing instruments, whilst bench top units have been dedicated to either GC/MS or LC/MS units. Many existing LC/MS systems are difficult to operate and all exhibit poor sensitivity. For example detection limits of 5-500ng in EI operation have been reported by Northington and co-workers (1990).

The poor performance of existing systems may be attributed to their "add on" design philosophy which results in unnecessarily long interfaces.

This paper describes the first benchtop, particle beam LC/GC/MS to use a complete system design approach to permit both the LC and GC interfaces to remain connected during operation in either mode. For probe work the particle beam interface is easily removed and the probe installed. The design provides a close coupled LC interface which improves sample throughput. The differential pumping of the interface is carried out using two ports per chamber in order to achieve balanced gas flow dynamics. This alone results in a doubling of sensitivity.

Detection limits for the particle beam interface below 5ng for EI+ mass spectrometer operation have been demonstrated. Indeed, full, library searchable spectra are routinely obtained from samples of 5ng. Excellent performance has also been observed in CI+ and CI- modes of operation. The CI results are presented together with EI spectra and detection limits for a variety of compounds.

LIBRARY SEARCHABLE EI SPECTRA

<table>
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5ng Caffeine
Volatility Enhancement of Nonvolatile Solutes by the Combination of a Heated Target and a Solvent-Depleted Particle Beam

*Edward W. Sheehan, Suhas Ketkar, and Ross C. Willoughby
Extrel Corporation, 575 Epsilon Dr., Pittsburgh, PA 15238

The introduction of solute into a mass spectrometer in the form of a solvent depleted solute particle beam has been of great interest the last 2-3 years. There have been observations that the temperature of the source plays an important role in the vaporization of the solute. For example, Jones et al.\(^1\) noted, that using a particle beam interface, that polystyrene oligomers of n=11 (mass 1202) and below were effectively vaporized and observed in the total ion chromatogram at a source temperature of 200 °C. While if the source temperature was increased to 315 °C, oligomers out to n=20 were observed in the total ion chromatograph.

One important performance characteristic of the particle beam interface is the observed volatility enhancement over other solid sample introduction techniques. One explanation for these results is related to the extremely small particle size generated with the thermal concentric nebulizer compared to other aerosol generation techniques. It is thought that the addition of surface energy in the aerosol generation process enhances the volatility of less volatile solute. Another explanation could relate to increased surface area per mass of an aerosol requires that more solute is at or near a surface. Since evaporation is a surface phenomena, the probability of forming intact gas phase solute molecules increases with increased surface area and the probability of thermal degradation decreases.

According to the First Law of Thermodynamics, the change in state (solid => vapor) is independent of path. One can separate the energy required to change a solid into a vapor, into a heat and work function. Heat is potentially destructive leading to the generation of pyrolysis products, while work (e.g. the increase in surface area of the sample) in turn increases the chance that the majority of solute molecules will be closer to the surface, thus increasing their chance of vaporization.

Solute vaporization on a flat surface such as a solids probe or on a moving belt requires heat to be transported from a heated surface, across the sample, to the surface of the sample. The solute trapped on the inside of the sample layer may be exposed to significant amounts of heat, potentially causing pyrolysis. The particle beam sample introduction; however, will result in the sample being transported directly to the heated surface with plenty of surface area for evaporation to occur. The more surface area associated with the sample, the greater the volatility enhancement. Electron micrograph measurements have shown thermal aerosols to have a much smaller average diameter than pneumatic aerosols.\(^2\)

A quadrupole mass spectrometer (differently pumped) equipped with a particle beam LC/MS interface (ThermaBeam, a thermal concentric nebulizer) from Extrel Corporation (Pittsburgh, PA) was used.

The present study was undertaken to study the effects of impacting solute particles on heated targets to enhanced the volatility of organic compounds. Three configurations were evaluated:

1. An ion source without a heated target.
2. A prototype heated target consisting of a 1/2-inch probe with a ribbon of nichrome (0.125-inch wide, by 0.325-inch long, by 0.01-inch thick). A DC current was passed through the ribbon until it glowed slightly red. The current was control with a variable transformer.
3. The DCI probe was a standard DCI probe for an ELQ-400 from Vacuumeetrics, Corp. (Ventura, CA). A current of 3 amps. was passed through the platinun wire.
The source temperature for all three configurations was 300 °C. The liquid chromatograph was operated at 0.5 ml/min. The mobile phase consisted of water and methanol (1/1) for all compounds except the C-60 and C-70 fullerenes (Buckyballs). The mobile phase consisted of toluene and methanol (1/1). The ThermaBeam LC/MS interface was optimized to give a 10/1 signal/noise from the flow injection of 5 ng (10ng/1μl) caffeine.

Conclusion The use of heated targets showed that intact molecular species could be formed and be subsequently ionized. There was no enhancement of the volatility of low molecular weight volatile compounds (e.g. caffeine and benzidine) but there was a 2-3X increase in the volatility and less pyrolysis fragments of relatively high molecular nonvolatile compounds.

References


EVALUATION OF A PARTICLE-BEAM LIQUID CHROMATOGRAPH/MASS SPECTROMETER

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An Extrel, Inc. (Pittsburgh, PA) Thermabeam particle-beam interface was connected to a Micromeritics, Inc. (Norcross, GA) liquid chromatograph and a VG Instruments, Inc. (Danvers, MA) high-resolution mass spectrometer. The connection to the mass spectrometer was made through the port previously used to install a moving-belt interface on the mass spectrometer. No modifications other than the use of an additional rubber o-ring and longer screws were needed to mate the two instruments.

Standard solutions of caffeine in methanol (3.41 pg/µL to 55.8 ng/µL) were prepared by serial dilution with each succeeding standard containing one-half of the concentration of caffeine in the previous solution. In addition, caffeine standards containing a constant concentration of an internal standard (theophylline or carbazole) were prepared. One series of standards contained 90.9 ng/µL of theophylline and from 11.1 pg/µL to 90.9 ng/µL of caffeine, and another series contained 20.3 ng/µL of carbazole and from 3.10 pg/µL to 50.7 ng/µL of caffeine.

Twenty-five µL injections of the standard solutions were made. The liquid chromatograph was operated isocratically using methanol at 0.4 mL/min. Under these conditions, there is no separation of caffeine, theophylline, or carbazole. Consequently, in this study, the liquid chromatograph serves only to introduce the sample to the particle-beam separator, and the method has the characteristics of flow injection. The mass spectrometer was operated in the electron-impact mode. The resolution was 5000 (2% valley) in the full scan (m/z 40 to 700) or selected-ion monitoring (SIM) mode. The scan rate was 2 s/decade in the full-scan mode and the cycle time was 1 s in the SIM mode.

Although the scan range extends over a wide range, exact mass measurements of the molecular ions of caffeine and theophylline gave excellent results, the deviation for caffeine (194.08038) being -7.5 ppm and that for theophylline (180.06485) being 0.7 ppm. The results of the analyses are listed in Tables 1, 2, and 3. Examination of Table 1 reveals that a good linear response is obtained for full scan (4.44 to 2272 ng of injected caffeine) or SIM (1.11 to 284 ng of injected caffeine) analyses. Lower amounts were not measured because theophylline contains an impurity that produces an interference in the m/z 194.0804 channel.

Examination of Table 2 shows that good linear response (77.4 pg to 1268 ng of injected caffeine) and a lower detection were obtained when carbazole was substituted for theophylline. However, for an injected mass of caffeine of 317 ng or greater, the area of the peak produced by the injected mass of 508 ng of carbazole was significantly reduced. Therefore, carbazole could not be used for internal standard calculations at high caffeine concentrations.

Examination of Table 3 reveals that although the linear response of caffeine in the absence of an internal standard is fairly good (% RSD = 31.2) over the wide range of injected mass of (85.1 pg to 1395 ng, a range factor of 16,385), there is a clear trend toward a reduction in the calculated response factor as the concentration of caffeine decreases. This supports the suggestion that as the concentration of an analyte decreases, the size of the particles produced in the interface decreases, and that the smaller particles are more readily pumped away and lost than larger particles. This effect is not as prevalent in the other runs because the constant high concentration of coeluting internal standard tends to maintain a more constant particle size as the concentration of the analyte decreases.
### TABLE I. DETERMINATION OF CAFFEINE IN THE PRESENCE OF THEOPHYLLINE

<table>
<thead>
<tr>
<th>Known amount injected, ng</th>
<th>Full Scan</th>
<th>Selected ion monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>External Std</td>
<td>Internal Std</td>
</tr>
<tr>
<td>2272</td>
<td>2148</td>
<td>2081</td>
</tr>
<tr>
<td>1138</td>
<td>1031</td>
<td>1096</td>
</tr>
<tr>
<td>606</td>
<td>487</td>
<td>516</td>
</tr>
<tr>
<td>254</td>
<td>201</td>
<td>234</td>
</tr>
<tr>
<td>142</td>
<td>158</td>
<td>120</td>
</tr>
<tr>
<td>71.0</td>
<td>87.5</td>
<td>81.8</td>
</tr>
<tr>
<td>39.8</td>
<td>31.9</td>
<td>31.6</td>
</tr>
<tr>
<td>17.8</td>
<td>23.7</td>
<td>25.7</td>
</tr>
<tr>
<td>8.88</td>
<td>7.89</td>
<td>6.83</td>
</tr>
<tr>
<td>4.44</td>
<td>4.06</td>
<td>4.33</td>
</tr>
<tr>
<td>2.22</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1.11</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*RF = 2.26 x 10^6 Relative standard deviation = 16.6%.

*RRF = 1.35; Relative standard deviation = 17.6%.  

*RF = 94; Relative standard deviation = 10.5%. 

*RRF = 2.17; Relative standard deviation = 10.1%. 

*NA = Not analyzed. 

*ND = Not detected.

### TABLE II. DETERMINATION OF CAFFEINE IN THE PRESENCE OF CARBAZOLE USING SELECTED ION MONITORING

<table>
<thead>
<tr>
<th>Known amount injected, ng</th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>External Std</td>
<td>Internal Std</td>
</tr>
<tr>
<td>1268</td>
<td>1718</td>
<td>NR</td>
</tr>
<tr>
<td>634</td>
<td>640</td>
<td>NR</td>
</tr>
<tr>
<td>316</td>
<td>393</td>
<td>NR</td>
</tr>
<tr>
<td>158</td>
<td>187</td>
<td>188</td>
</tr>
<tr>
<td>75.3</td>
<td>78.2</td>
<td>85.8</td>
</tr>
<tr>
<td>35.8</td>
<td>36.0</td>
<td>40.8</td>
</tr>
<tr>
<td>10.6</td>
<td>20.1</td>
<td>19.8</td>
</tr>
<tr>
<td>6.81</td>
<td>9.69</td>
<td>10.2</td>
</tr>
<tr>
<td>4.68</td>
<td>4.89</td>
<td>4.86</td>
</tr>
<tr>
<td>2.41</td>
<td>2.08</td>
<td>2.22</td>
</tr>
<tr>
<td>1.24</td>
<td>0.899</td>
<td>1.11</td>
</tr>
<tr>
<td>0.619</td>
<td>0.615</td>
<td>0.704</td>
</tr>
<tr>
<td>0.310</td>
<td>0.278</td>
<td>0.253</td>
</tr>
<tr>
<td>0.169</td>
<td>0.132</td>
<td>0.133</td>
</tr>
<tr>
<td>0.071</td>
<td>0.074</td>
<td>0.051</td>
</tr>
</tbody>
</table>

*RF = 493; Relative standard deviation = 17.0%.  

*RRF = 0.408; Relative standard deviation = 11.4%. 

*RF = 386; Relative standard deviation = 21.1%. 

*RRF = 0.490; Relative standard deviation = 10.0%. 

*NR = Not reported because low values of the area of the internal standard peaks produced low values of the RRF.

### TABLE III. DETERMINATION OF CAFFEINE IN THE ABSENCE OF AN INTERNAL STANDARD USING SELECTED ION MONITORING

<table>
<thead>
<tr>
<th>Known amount injected, ng</th>
<th>Calculated amount, ng</th>
</tr>
</thead>
<tbody>
<tr>
<td>1399</td>
<td>2180</td>
</tr>
<tr>
<td>896</td>
<td>1100</td>
</tr>
<tr>
<td>348</td>
<td>531</td>
</tr>
<tr>
<td>134</td>
<td>205</td>
</tr>
<tr>
<td>87.2</td>
<td>87.1</td>
</tr>
<tr>
<td>43.6</td>
<td>40.2</td>
</tr>
<tr>
<td>21.8</td>
<td>20.0</td>
</tr>
<tr>
<td>10.9</td>
<td>10.3</td>
</tr>
<tr>
<td>8.45</td>
<td>4.56</td>
</tr>
<tr>
<td>2.72</td>
<td>2.69</td>
</tr>
<tr>
<td>1.38</td>
<td>1.05</td>
</tr>
<tr>
<td>0.681</td>
<td>0.479</td>
</tr>
<tr>
<td>0.341</td>
<td>0.235</td>
</tr>
<tr>
<td>0.170</td>
<td>0.123</td>
</tr>
<tr>
<td>0.0681</td>
<td>0.0837</td>
</tr>
</tbody>
</table>

*RF = 428; Relative standard deviation = 31.2%.
Two major deficiencies of the particle beam (PB) interface have become apparent: 1) transport efficiency of analytes through the particle beam interface has been determined to be as low as 12% [1]; 2) transport efficiency is a function of analyte concentration, decreasing at low analyte concentration, which results in non-linear calibration curves. Both of these deficiencies can be corrected through the use of "carriers", compounds added to the eluent to enhance the transport efficiency of the analytes through a wide dynamic range. Ammonium acetate [2] has been employed as a mobile phase additive to improve analyte transport, as has the isotopically labelled analog of the analyte of interest [3].

In the present work the structural similarity between a class of analyte and a generic "carrier" is exploited to enhance the transport efficiency of a number of compounds within the class through the PB interface. For the phenylurea herbicides, diuron, linuron, and neburon, 2.9 ng/μL of phenylurea (PHU) is added as the carrier pre-column to the mobile of 68% acetonitrile/32% water. The calibration curves for diuron in Figure 1 show that without the PHU carrier a non-linear curve is obtained (a), but with the PHU carrier (b) the curve is linear. The Hewlett-Packard 5988A mass spectrometer is operated in methane enhanced negative ion selected ion monitoring (SIM) mode. The detection limit for diuron improves from 2 ng on-column without the PHU carrier to 160 pg on-column in the presence of the PHU carrier.
For the chloroalkoxy acid herbicides phenylacetic acid (PAA) was used as the generic carrier for the compounds 2,4-D, 2,4,5-T, and Silvex. Using 1.7 ng/μL PAA in the mobile phase of 70% methanol and 30% aqueous 1% acetic acid produced the linear calibration curves shown in Figure 3. The detection limit for 2,4-D was 1000 pg injected on-column when the mass spectrometer was operated in methane enhanced negative ion SIM. The pH of the injected herbicide solution must be adjusted to 2.8 to assure that the herbicides are in the neutral acid form.
PARTICLE BEAM LC/MS INTERFACED TO HIGH-FLOW-RATE, HIGH-WATER-CONTENT SEPARATIONS
R.J. Seymour, Rhone-Poulenc Ag Company, Research Triangle Park, NC, 27709

Introduction

The particle beam LC/MS interface is usually limited to mobile phase flow rates of 0.8 mL/min or less depending on the design. Also, the sensitivity is adversely affected when the amount of water in the mobile phase is greater than about 50%. Stream splitting and narrow bore columns have been used to overcome the problem of high flow rates. Post-column addition of strong organic solvent has been used to improve sensitivity when the mobile phase water content is high. The technique presented here is an alternative to those solutions and has the advantage of not splitting away or diluting the analyte and can be used with standard sized columns at relatively high flow rates.

How the trap and transfer technique works

A diagram of the peak trap and transfer device is shown below. A sample is chromatographed using the mobile phase supplied by Pump #1. This mobile phase may have a relatively high flow rate and water content as required by the chromatographic separation. When there are no peaks to be sent to the particle beam interface, the effluent passes from the UV detector through the six port valve to waste. A second pump delivers strong organic solvent, typically methanol or acetonitrile, to the particle beam interface via the trap column at a flow rate which is compatible with the interface. When a peak of interest is detected by the UV detector, the six port valve is switched such that the peak is passed onto the trap column while the mobile phase from Pump #2 goes directly to the particle beam interface. When the peak has passed completely onto the trap column, the valve is switched back to the initial position causing the peak to be flushed from the trap by the strong organic mobile phase from Pump #2. This stream, now containing the analyte, is directed to the particle beam interface and mass spectrometer where the mass spectrum of the peak is obtained.

Identification of an Herbicide Impurity

A small impurity peak eluting just before a large active ingredient peak in an herbicide sample needed to be identified. A flow rate of 3.0 mL/min and a mobile phase containing 87% water with 1% acetic acid/13% acetonitrile was required to separate this pair of peaks in less than about 20 minutes on a Whatman Partisil column (25 x 0.46 cm, 10m). A narrow bore column and other mobile phases were tried but did not separate these peaks adequately. Therefore, to obtain a spectrum of the impurity peak, it was necessary to use the peak transfer technique. The small impurity peak was trapped on a 3 cm Brownlee C-8 column. It was then flushed from the trap with 100% acetonitrile at 0.8 mL/min. This served to refocus the peak and transfer it to the particle beam interface. The impurity was identified from the NBS Library as 2,4-dichlorophenoxyacetic acid or 2,4-D.
Confirmation of a Sugarcane Extract Residue

Sometimes a peak needs to be identified without changing a given set of chromatographic conditions. A peak observed in a sugarcane residue method was thought to be acetyl sulphanilamide. This needed to be confirmed by LC/MS. The residue method used a mobile phase containing 89% aqueous ammonium acetate and 11% acetonitrile at 1.0 mL/min. The column used was a B&J OD5 (25 x 0.46 cm, 5m). Figure 6 shows the ion chromatogram obtained when the peak of interest was transferred to the particle beam from the trap column using a stream of 100% acetonitrile at 0.8 mL/min. The identity of the residue peak was confirmed by comparison of the retention time and mass spectrum obtained to that of a standard reference material.

Instrumentation used

- VG Trio-2 quadrupole MS with VG particle beam interface
- Hitachi L-6200 low pressure gradient HPLC pump (Pump #1)
- Waters M6000A HPLC isocratic pump (Pump #2)
- Waters 990+ Diode Array UV detector with high pressure cell
- Rheodyne 7000 six port valve (Have also used two 7125 injector valves in a slightly different configuration)
Development and Application of Phase-System Switching Techniques used for Particle Beam-Liquid Chromatography/Mass Spectrometry

Jill Guthrie, Gil Radolovic
Midwest Research Institute, 425 Volker Boulevard, Kansas City, MO, 64110

Introduction

The liquid chromatographic conditions required to successfully separate mixtures of highly polar compounds typically include mobile phases composed of over 50% water at flow rates of 1 ml/min or greater. However, sensitivity routinely decreases for many compounds when performing particle beam LC/MS under these conditions.

Loss of sensitivity can be minimized when performing particle beam LC/MS by using phase-system switching techniques such as coupled column chromatography (CCC). Coupled column chromatography has been used routinely in the past by liquid chromatographers, but it has been only recently that the benefits of it have been applied to LC/MS technology (1).

In the CCC technique, the LC peak of interest is diverted or "heart-cut" onto a secondary trapping column as it elutes from the primary chromatography column. After the peak has been isolated on the secondary column, phase-system switching is then performed using a multi-port valve and an auxiliary pump. The analyte is flushed from the secondary column into the particle beam interface with solvents and flow rates which are more compatible with the PB-LC/MS system.

MRI has designed a CCC PB-LC/MS system and has successfully applied the technology to the identification of polar constituents present in commercial pesticides, drug formulations, and grains.

Experimental

Certain characteristics of the primary LC chromatography are necessary for successful heart-cutting. Peak resolution in the vicinity of the peak of interest should be optimized in order to allow selective heart-cutting of only the peak of interest from the chromatogram. In addition, the retention time of the peak of interest should be reproducible, because retention times are used to determine the times for heart-cutting.

Any standard LC column can be used with its appropriate flow rate for the primary column. Common LC solvents such as water, methanol, and acetonitrile can be used to develop the chromatography. Although not applied in this work, it has also been shown that non-volatile buffer systems can be used (2).

The secondary trapping column should retain the heart-cut as a narrow band on the front of the column in order to avoid peak broadening. The solvent and the flow rate used to flush the secondary column should be compatible with the particle beam interface. A volatile buffer such as ammonium acetate can be added to the flushing solvent.

Results

The CCC PB-LC/MS system developed was used to isolate constituents of a methanolic flax seed extract and obtain spectra for them. The primary chromatography separation developed, required a linear methanol/water gradient beginning at 100% water and ending with 100% methanol in 60 minutes to resolve the very polar constituents of the extracts.

In order to evaluate the performance of the CCC PB-LC/MS system, a flax seed extract was spiked with 50 ng/ul each of five phenolic acids commonly found in cereal grains (Figure 1). 20 ul of this spiked sample were injected onto the primary LC column and the phenolic acid peaks were heart-cut. Each heart-cut LC peak resulted in a full scan PB-LC/MS peak with a minimum signal-to-noise ratio of 10 to 1 (Figure 2).
Detection and identification of the phenolic acids would have been difficult without the ability to heart-cut the selected peaks of interest which eluted in the early portion of the LC separation.

1. 4-hydroxybenzoic acid
2. 4-hydroxy-3-methoxybenzoic acid
3. 3,5-dimethyl-4-hydroxycinnamic acid
4. 4-hydroxy-3-methoxycinnamic acid
5. Nordihydroguaiaretic acid

![Figure 1. LC Chromatogram of a Spiked Flax Seed Extract](image1)

![Figure 2. CCC PB-LC/MS Chromatogram and Mass Spectrum of a 1ug Heart-Cut of 4-hydroxybenzoic acid (Mol. wt. 138)](image2)

**Acknowledgements**

This work was partially funded by the National Cancer Institute, Contract No. N01-CN-05281-01.

The authors also wish to acknowledge Debbie Alexander and Winona Arnold for preparing the flax seed extracts and developing the primary column chromatography used for that application.

**References**

ADVANTAGES OF PARTICLE-BEAM SAMPLE INTRODUCTION FOR ANALYSIS OF THERMALLY SENSITIVE NATURAL PRODUCTS BY MASS SPECTROMETRY

William F. Haddon and Leslie A. Harden, Western Regional Research Center, USDA 800 Buchanan St., Albany, CA. 94710

Introduction. The particle beam (P/B) liquid interface developed by Willoughby, Winkler and Browner utilizes a combined aerosol generator-nebulizer and momentum separator to produce a stream of solute particles which are subsequently ionized following their impact on the surface of the ion source. The P/B separator provides a desirable interface for liquid chromatography in that mass spectra (EI, CI and LSIMS) can be obtained under conditions which allow direct comparison to literature and library spectra for many compounds. Additionally, the P/B enrichment device extends the lower limit of volatility below that of GC and direct probe analysis methods.

We report here the electron ionization (EI) mass spectra of a series of condensation products between dimedone and substituted phenyl benzopyrans for which markedly superior spectra were obtained by direct liquid introduction using a commercially available P/B enrichment interface.

Results. Figure 1 lists four compounds which posed difficulties for direct introduction probe (DIP) analysis because of their thermal instability. Figure 2 shows the problem of obtaining a structurally representative EI mass spectrum of IV using an "in source" DIP. The molecular ion of IV was present only transiently at scan 21, along with structurally significant fragment ions at 273 and 274 daltons. Ions arising from thermal decomposition dominate the spectra following scan 21 and these non-representative spectra comprise the bulk of the volatilization profile; the spectrum at scan 26 contains no measurable molecular ion, and structurally diagnostic peaks have been lost below more abundant ions, such as the 257d peak, which apparently arises from thermal decomposition prior to volatilization.

Figure 3 shows the EI spectra obtained from about 500 ng of the same compound, IV, introduced via the particle beam interface using chloroform as solvent. The nebulizer tip was at 160 deg C, the desolvation temperature was 95 deg., and the ion source temperature was 180 deg. The molecular ion (502d) is prominent over the full range of the flow injection for P/B sample introduction. The only significant fragment ions we observe are the direct cleavage and McLafferty rearrangement ions at 273 and 274d respectively. In contrast to the DIP spectra, the P/B spectra are useful for assignment of molecular structure. The slightly lower molecular ion abundance of the P/B EI spectrum probably reflects the use of the quadrupole vs. magnetic analyzer.

Compounds I-III behaved similarly for direct probe analysis, showing extensive decomposition. In all cases P/B sample introduction yielded suitable EI spectra routinely. With the DIP improved results could be obtained by careful control of source temperature and heating rate. Figure 4 illustrates the improvement in the EI spectrum of IV using a faster DIP heating rate.

Discussion. The improved spectra of thermally sensitive compounds by P/B sample introduction probably reflects a relatively higher rate of heat transfer to the sample surface compared to the DIP. There is evidence for the importance of rapid heating in the literature in previous studies by Friedman et al. in which the activation energies for competing thermal decomposition and volatilization processes for thyrotrop releasing hormone (TRH, pyrolidinonecarboxylamide-His-Pro-NH₂) were shown to be different. For TRH, thermal decomposition proceeds with lower activation energy compared to volatilization; thus the volatilization process will predominate at higher sample temperature.

For the experimental conditions used in this work, the P/B aerosol generator should produce a narrowly-distributed range of particle sizes with an average of about 100 μm. diameter. For a roughly Gaussian distribution of sample in the 15 ul injection volume (see TIC curve of Figure 3), the sample concentration over the central portion of the curve is about 350 ng for a 500 ng injection. Aerosol formation should yield about 1.4 x 10⁷ droplets, assuming minimal recombination in the desolvation region, resulting in particle thickness of about 0.4 μm, based on spherical particles and complete removal of solvent. In comparison, a 10 ng sample deposited on the direct probe will have a sample thickness about two orders of magnitude lower, roughly 0.025 μm.
Thus the high temperature of the ion source probably accounts for the rapid heating rate for impacting particles rather than increased surface area or reduced sample thickness compared to the DIP. Of course very high heating rates can be achieved on a direct probe, for example by use of Curie-point probes. The advantage of P/B sample introduction is that the rate of heating the sample, which is determined largely by the impact surface temperature, and the rate of sample consumption, which is determined by the LC peak width or flow injection volume, are independently established. This independence of heating rate and rate of sample consumption allows spectra to be recorded at acceptably slow scan rates on the mass spectrometer even when the heating rate must be increased to minimize the effect of thermal decomposition. Of course molecular ion abundances are significantly reduced with increased temperature in many cases, and this factor will limit the utility of the spectra for some compounds.

Conclusions. For thermally sensitive natural product samples, introduction into the mass spectrometer directly in liquid solution via a particle beam interface can produce superior spectra compared to measurements with an in source direct probe. Using a high temperature for the impact surface, where volatilization occurs, will lead to improved spectra in cases where competing thermal decomposition reactions have lower activation energy.

Acknowledgement. Drs. L. Jurd and C. Elliger, WRRC, provided samples used in the work.

Analysis of Some Derivatives of Steviol by Particle Beam LC/MS

Extracts of the plant Stevia rebaudiana are used in several countries as sweeteners. The major sweet compounds are the ent-kaurene glycosides stevioside and rebaudioside A. In addition another structurally related sweet glycoside, rubusoside, is found in a sweet tea product consumed in the People's Republic of China. These compounds all contain a common aglycone, steviol.

Steviol (I), when metabolically activated has been found to be mutagenic and a number of these in vitro metabolites have been identified and studied (1) by GC/MS and GC/MS/MS. In addition another study (2) has characterized steviol and its metabolites by DCI/MS using ammonia and dimethyl ether as reagent gases.

In the current work a series of analogs of steviol was separated by reversed phase HPLC and analysed via the Particle Beam LC/MS interface in order to evaluate the suitability of this technique for the study of steviol metabolism.

**Experimental:** Steviol, a series of its in vitro metabolites, and their methyl esters were prepared as described previously (1). The compounds were chromatographed using a HP 1090L HPLC on a 250mm x 2.1mm Hypersil C-18 column with the following gradient system: solvent A- 0.01M NH4Ac (pH 3.5), solvent B- ACN:0.01M NH4Ac (80:20), Initial-(A:B) 50:50 to 40:60 at 6 min, to 25:75 at 10 min, to 20:80 at 12 min, then hold to 20 min. The flow rate was 0.4 mL/min. The eluting compounds were first passed through a diode array detector (220 nm) connected in tandem with the particle beam LC/MS interface. The MS were obtained with a HP 5989A LC/MS under the following conditions: El mode- scan 60-400; Ammonia PCI mode- scan 100-500, source pressure 0.8 torr; Ammonia NCI mode- scan 120-500, source pressure 0.8 torr; Methane PCI mode- scan 100-500, source pressure 1.0 torr; Dimethyl Ether PCI mode- scan 140-500, source pressure 1.4 torr. In all modes the source temperature was held at 275 C, the desolvation chamber temperature was 55 C and the helium gas flow was set to 60 psi.

**Results and Discussion:** The chromatographic system developed is capable of resolving all of the derivatives with the exception of dehydrosteviol A and B and their corresponding methyl esters. The chromatogram shown in II is typical of the results obtained for an 8 uL injection of a mixture containing approximately 100 ng/component/uL of 14 compounds. The UV chromatogram is shown below it (III). Because most of these compounds show poor UV absorbance (with exception of the 15-oxosteviol), the use of LC/MS proves to be a superior detection method at low concentrations. In general most of the compounds studied showed a detection limit of ca. 100 ng on column under El and PCI (methane and ammonia reagent gases) conditions. The methyl esters gave slightly better results. The use of dimethyl ether as a reagent gas in PCI was also investigated and detection limits were only half those of ammonia or methane. The spectra obtained for these compounds were found to be consistent with those reported earlier (1,2). A clear advantage in using LC/MS for the routine analysis of these compounds is that derivatization to enhance volatility is not required to obtain useful detection limits. Indeed, the slight improvement in sensitivity for the methyl esters could be due as much to their eluting at a higher organic solvent concentration (3) as it could be to any enhancement in their volatility.

FIGURE I
STEVIOL

PARTICLE BEAM EI MODE
TOTAL ION CHROMATOGRAM

FIGURE II

UV CHROMATOGRAM (220nm)

FIGURE III

TIME (Minutes)

Legend:
2- Steviol Norketone
4- 17-OH Steviol
1a- 15-OH Steviol Methyl Ester
8- Steviol Epoxide
3a- 15-Oxosteviol Methyl Ester
4a- 17-OH Isoateviol Methyl Ester
0- Steviol
5a- Steviol Epoxide Methyl Ester
7- Dihydrosteviol A
9- Dihydrosteviol B
18a- Isosteviol
7a- Dihydrosteviol A Methyl Ester
8a- Dihydrosteviol B Methyl Ester
9a- Isosteviol Methyl Ester
AN INVESTIGATION OF ION ABUNDANCE ENHANCEMENTS FOR THE PARTICLE BEAM LC/MS ANALYSIS OF ETHYLENETHIOUREA (ETU) IN FOOD SAMPLES

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*Dept. Environ. Biochem., University of Hawaii, Honolulu, HI 96822, USA.

It is well known (1) that particle beam LC/MS interfaces inherently display ion abundance enhancement effects where the intensity response for a particular compound is typically enhanced by the presence of a coeluting compound. This coelution effect is detrimental when using isotope-labelled internal standards for quantitative analyses of target compounds since there is no rule of thumb method for predicting the contribution to the target response due to coelution.

Recently we demonstrated the use of particle beam LC/MS for the identification and quantification of ethylenethiourea (ETU), a fungicide metabolite and probable human carcinogen, in spiked and natural food samples with detection limits of 5ppb in the crop (2).

Figure 1 shows the on-column, PB LC/MS detection of 10(±5)ppb ETU in a papaya extract and the simultaneous internal standard response for 10ng of 13C ETU. At these low levels of detection (1-10ng ETU), and for comparable amounts of target and internal standard, it was reported that the effects of coelution in the VG LINC™ PB LC/MS interface were in fact negligible. In this communication, we present some data on the ion abundance enhancement of ETU for varying amounts of selected internal standards and a range of interface desolvation chamber temperatures.

All data were obtained from 10μl loop injections using a VG LINC™ PB LC/MS interface incorporating a Hildebrand grid nebulizer and a mobile phase composition of 50% CH3CN/water at 0.5ml/min. Unless otherwise stated, the desolvation chamber temperature (measured in the gas flow) was maintained at 30°C. MS measurements were made using a VG TRIO-2000 quadrupole system operating in positive ion El mode with a source temperature of 200°C and scanning the mass range 70-300amu in 1 second. ETU response was determined by the area under the chromatogram peak for the molecular ion m/z = 102 (m/z = 103 for the 13C-ETU internal standard). All data points are averages of 3 determinations (RSD ≤ 10%).

Figure 2 shows the coelution enhancement of 10ng of native 12C ETU due to the addition of increasing amounts of 13C ETU internal standard. Here, the enhancement factor is simply the ratio of the 12C response with internal standard to the 12C response with no internal standard. From a similar analysis, Figure 3 shows the coelution enhancement of 10ng of 12C ETU by the addition of increasing amounts of aminotriazole (MW = 84). From these enhancement relationships, it is seen that for comparable amounts of native compound and internal standard (in this case for 13C ETU and aminotriazole additions of ≤ 25ng) the coelution enhancement is negligible, as was previously reported (2). However, as the amount of internal standard is increased beyond 25ng, the enhancement factor increases dramatically such that the response to 10ng of native 12C ETU is enhanced by a factor of 14 by the addition of 500ng of 13C ETU. In addition to this internal standard concentration dependence, it appears that the enhancement is also dependent on the coeluting compound where it is seen that 13C ETU has an approximately 4 times greater enhancement effect than aminotriazole for 500ng additions.

During the course of these measurements it was observed that the desolvation chamber temperature had a strong influence on the reproducibility of these data. Figure 4 shows the effect of desolvation chamber temperature on the enhancement factor for 10ng of 12C ETU coeluting with 50ng of 13C ETU. Thus, it is seen that, under these
coeluting conditions, the enhancement factor increases approximately 4-fold as the temperature is increased from 25 to 50°C.

In the context of quantitative PB LC/MS analyses, these results serve to illustrate the need for careful calibration for the choice of internal standard, concentration and experimental conditions.

![Figure 1](image1.png)  
**Figure 1.** On column detection of 10ppb ETU in a papaya fruit extract.

![Figure 2](image2.png)  
**Figure 2.** The response enhancement of 10ng of $^{12}$C ETU with coeluting $^{13}$C ETU.

![Figure 3](image3.png)  
**Figure 3.** The response enhancement of 10ng of $^{12}$C ETU with "coeluting" aminotriazole.

![Figure 4](image4.png)  
**Figure 4.** Temperature dependence of the response enhancement of 10ng $^{12}$C ETU ($^{13}$C ETU = 50ng).

**References**


Surfactant Analysis by Ion Chromatography/
Particle Beam Mass Spectrometry

James N. Alexander, Chad J. Quinn. Rohm and Haas Company, Research Laboratoires, Spring House, PA 19477

Introduction:
Ion chromatography/mass spectrometry (IC/MS) has been investigated, using the particle beam interface, as an analytical technique for the analysis of anionic and nonionic surfactants. Ion pair chromatography is an excellent technique for separating hydrophobic anions. The surfactants were separated on a hydrophobic resin-based column utilizing acetonitrile as the organic modifier and ammonium hydroxide as the ion pair reagent. Most ion pair reagents are non-volatile, therefore, the use of ammonium hydroxide makes this technique compatible with the mass spectrometer. A main advantage of this technique is that co-eluting surfactants can be identified, whereas, with conductivity and UV detection this is difficult.

IC/MS was used to detect and identify surfactants in commercial laundry detergents and shampoos. Analyses were performed using both electron (EI) and chemical ionization (CI). Methane CI readily produced protonated molecular ions on some surfactants, and combined with structural information obtained from EI analyses, identification of alkylbenzene sulfonates and alkylphenol polyethoxylates is possible. Alkyl sulfates also produced molecular ions using negative ion analyses and were used to confirm their presence in shampoos and detergents.

Experimental Conditions:
The experiments were performed on a Hewlett Packard (HP) 5988 quadrupole mass spectrometer. The HP particle beam LC/MS interface was used to couple the mass spectrometer to a HP Model 1090 high-performance liquid chromatograph. The instrument was operated in the full scan mode for all experiments.

The surfactants were separated on a Polymer Laboratories PLRP-S (5 micron packing, 100 Å pore size, 15 cm x 4.6 mm ID) column. The separations were performed using a gradient method. The flow rate was 0.5 mL/min and the injection volume was 20 microliters. Mobile phase A was 5% acetonitrile, 10 mM ammonium hydroxide and mobile phase B was 95% acetonitrile, 10 mM ammonium hydroxide. The gradient conditions are listed below.

<table>
<thead>
<tr>
<th>Time</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>85% A / 15% B</td>
</tr>
<tr>
<td>15 min</td>
<td>30% A / 70% B</td>
</tr>
<tr>
<td>20 min</td>
<td>100% B</td>
</tr>
<tr>
<td>25 min</td>
<td>85% A / 15% B</td>
</tr>
</tbody>
</table>

The following parameter settings were used for the mass spectrometer. The source temperature and pressure were 275 °C and 0.8 torr (methane), respectively. The electron multiplier was 2300 kV and the scan range was 65 - 900 daltons. The
following parameter settings were used for the particle beam interface. The desolvation chamber was set at 65 °C. The nebulizer helium pressure was 30 psi and the capillary position was extended approximately 0.9 mm.

Results and Discussion:
Anionic and nonionic surfactants are widely used in industry. Many chromatographic techniques have been used to separate and determine surfactants in a variety of samples. Today, high performance liquid chromatography (HPLC) and ion chromatography (IC) are the leading techniques used for surfactant analysis. Most often surfactant identifications are made by comparing retention times to standards. However, similar surfactants can co-elute and make identifications difficult when using UV or conductivity detection. Using IC/MS (particle beam interface) we were able to identify co-eluting surfactants by their mass spectra. We applied this chromatographic technique for the analysis of ionic and nonionic surfactants in laundry detergents and shampoos.

Both EI and Cl ionization techniques were employed with methane as the reagent gas. Negative Cl produced characteristic mass spectra for the alkyl sulfates. The negative charged parent ions were present for each of the alkyl sulfates from C_{10} to C_{16}. In most cases the base peak in the negative ion mass spectra was the loss of oxygen. Positive Cl produced protonated molecular ions with little fragmentation for the alkylbenzenesulfonates from C_{10} to C_{13}. Additional structural information was obtained by EI analyses of the alkyl sulfates and alkylbenzenesulfonates. EI analysis of the alkyl sulfates produced mass spectra with the classical low mass hydrocarbon ion series. With these characteristic mass spectra and retention time comparisons of standards we analyzed Vidal Sassoon shampoo and Ultra Tide laundry detergent.

Negative ion and EI analyses of Vidal Sassoon shampoo showed that the C_{12}, C_{14} and C_{16} alkyl sulfates were present. No presence of alkylbenzenesulfonates was observed. The analyses of Ultra Tide confirmed the presence of C_{14} and C_{16} alkyl sulfates and the linear C_{12} alkylbenzenesulfonate. The C_{14} alkyl sulfate and the linear C_{12} alkylbenzenesulfonate co-elute under these chromatographic conditions. With EI and Cl mass spectra, interpretation of the co-eluted peaks was possible, allowing for the determination of the presence of both the sulfates and the sulfonate.

Positive Cl was also useful for identifying nonionic surfactants specifically Triton X-100. Positive Cl generated molecular ions thus allowing the various homologs to be identified. We did not observe this surfactant in either the laundry detergent or shampoo.

Conclusion:
Particle beam ion chromatography mass spectrometry has proven it's ability to generate characteristic mass spectra for the identification of ionic and nonionic surfactants. Employing EI and CI, both positive and negative ion detection, the characterization and identification of surfactants in commercial laundry detergents and shampoos was possible.
Quantification of Tryptophan and Kynurenine Pathway Metabolites by Particle Beam-LC Negative Chemical Ionization-MS


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The kynurenine pathway is the major route of tryptophan metabolism producing a number of intermediates which may be pharmacologically active in the central nervous system (1). Renewed interest in the kynurenines has resulted in the need for improved methods for quantifying them in biological fluids and tissues. A number of liquid chromatographic methods with UV absorbance, fluorescence, or electrochemical detection and gas chromatographic methods with electron capture or mass spectrometric detection have been described (2). Particle beam-LC/MS is particularly well suited to analyses of chemically similar compounds in complex aqueous matrices. Mass analysis provides additional specificity to quantification as well as the ability to use stable isotope labeled analogs as internal standards or as tracers in biological systems.

We have developed a method for the quantification of L-tryptophan (TRP) and its metabolites L-kynurenine (KYN), 3-hydroxykynurenine (3HK), 3-hydroxyanthranilic acid (3HAA), quinolinic acid (QA), kynurenic acid (KA), anthranilic acid (AA), and xanthurenic acid (XA). These compounds contain multiple functional groups but each contains at least one carboxyl function. All of the above were quantitatively esterified with pentafluorobenzyl bromide to increase electron capturing ability and enhance negative chemical ionization (NCI) mass spectrometric response.

Samples of a mixed standard solution in 1 M HCl were freeze-dried and each was derivatized with 80 µL PFB-Br (5% v/v in acetonitrile) and 20 µL diisopropylethylamine, at 70°C for 15 minutes (3). Derivatized samples were taken to dryness under a stream of nitrogen and reconstituted in acetonitrile. The resulting solutions were transferred to autosampler vials and analyzed by LC/MS.

Liquid chromatography was carried out on a HP 1050 series LC system equipped with a 5 µm ODS Hypersil column, 100 mm x 2.1 mm id at a flow rate of 0.4 mL/min. Gradient elution was used. A mixed mobile phase (28% 0.1 M ammonium acetate:32% methanol:40% acetonitrile adjusted to pH 5 with acetic acid) was maintained for 6.5 minutes to maximize separation of KYN and TRP, followed by a linear gradient to 100% acetonitrile at 16.5 min to 20 min. A post-run of 10 min was used to allow the column to re-equilibrate with the initial mobile phase.

LC eluent was introduced into the mass spectrometer via a HP 59980B Particle Beam Interface. Helium nebulized pressure was 50 psi and interface temperature maintained at 48°C. NCI-MS was performed on a HP 5999 mass spectrometer with methane as reagent gas with source pressure of 1 torr. Source and quadrupole temperatures were 250°C and 100°C, respectively. Spectra of the PFB derivatives of tryptophan and metabolites were obtained by scanning from m/z 100 to 800 at 0.40 scans/sec. All spectra exhibit prominent [M - PFB]~ ions. These characteristic ions dominate the NCI spectra of the derivatives except for that of 3HK (exhibits substantial fragmentation).

18O labeled isotopomers of QA, KYN, TRP, KA, 3HAA, AA, 3HK, and XA were prepared by exchange in 1 M HCl/H2[18O] at 70°C for 24 hours. Samples of each preparation were combined, derivatized, and analyzed by LC/MS. Oxygen exchange of QA gave primarily m/z 354 representing exchange of all four carboxylate oxygens. 18O-QA was not detected. TRP, KA, 3HAA, AA, and XA had predominantly [18O]3 ions; both carboxylate oxygens were exchanged. KYN and 3HK produced primarily the [16O]3 isotopomers with exchange of the ketone group as well as the carboxylate oxygens. Most conveniently, as has been reported previously (4), [16O]2, [16O]3, or [18O]4 labeled...
Isotopomers retained their isotope label upon derivatization and exhibited prominent \([M - PFB]^-\) ions in their NCI spectra. Thus, a useful internal standard for each target analyte was readily prepared.

A series of samples containing the PFB derivatives of QA, KYN, TRP, KA, 3HAA, AA, and 3HK in acetonitrile were analyzed by LC/MS monitoring ions corresponding to \([M - PFB]^-\). Signal:noise for 25 pg injected ranged from 100:1 for TRP to 4:1 for 3HK. The PFB derivative of 3HK shows significant fragmentation and may not be particularly well suited to NCI quantification.

Results are summarized in Table 1. The LC method provided adequate resolution with peak widths of a half minute. Derivatization with pentafluorobenzyl bromide allowed detection of these compounds by NCI mass spectrometry with sensitivities in the mid picogram range.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Derivative</th>
<th>RT* (min)</th>
<th>m/z 16(^{16})O</th>
<th>m/z 16(^{18})O</th>
<th>Signal:Noise 25 pg</th>
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<tbody>
<tr>
<td>Quinolinic Acid</td>
<td>QA(PFB)(^2)</td>
<td>3.9</td>
<td>346</td>
<td>354</td>
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<tr>
<td>L-Kynurenine</td>
<td>KYN(PFB)(^2)</td>
<td>5.1</td>
<td>387</td>
<td>393</td>
<td>20:1</td>
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<tr>
<td>L-Tryptophan</td>
<td>TRP(PFB)(^2)</td>
<td>6.0</td>
<td>383</td>
<td>387</td>
<td>111:1</td>
</tr>
<tr>
<td>Kynurenic Acid</td>
<td>KA(PFB)(^2)</td>
<td>9.4</td>
<td>358</td>
<td>372</td>
<td>18:1</td>
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<tr>
<td>3-Hydroxyanthranilic Acid</td>
<td>3HAA(PFB)(^2)</td>
<td>11.9</td>
<td>333</td>
<td>336</td>
<td>30:1</td>
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<tr>
<td>Anthranilic Acid</td>
<td>AA(PFB)(^2)</td>
<td>13.3</td>
<td>316</td>
<td>320</td>
<td>47:1</td>
</tr>
<tr>
<td>3-Hydroxykynurenine</td>
<td>3HK(PFB)(^3)</td>
<td>13.4</td>
<td>583</td>
<td>589</td>
<td>4:1</td>
</tr>
<tr>
<td>Xanthurenic Acid</td>
<td>XA(PFB)(^3)</td>
<td>14.8</td>
<td>584</td>
<td>588</td>
<td>-----------------</td>
</tr>
</tbody>
</table>

* LC solvents: A = 28% 0.1 M NH\(_4\)Ac, 32% CH\(_3\)OH, 40% CH\(_3\)CN adjusted to pH 5 with HAc; B = 100% CH\(_3\)CN

LC gradient: 100% A for 6.5 min linear gradient to 100% B at 16.5 min 100% B to 20 min; Post time = 10 min

REFERENCES

A particle beam interface was constructed after the design of Extrel's ThermaBeam™ interface (Extrel Corp., Pittsburgh, PA) and retrofitted to a Finnigan 4021 quadrupole mass spectrometer equipped with a Teknivent Vector II data system (Teknivent Corp, Maryland Heights, MO.). This instrument is currently used in structure elucidation studies in Drug Metabolism Research. Extrel's particle beam design was chosen because we believe it is superior in performance to other commercially available particle beam interfaces. Permission to use this design was obtained from Extrel.

The objective was to provide: 1) an LC/MS screening tool that would provide superior structural information than thermospray spectra and 2) an interface that would also easily adapt to existing chromatographic separations developed off-line. Particle beam LC/MS was chosen because it is capable of electron impact, chemical ionization, and other desorption ionization techniques without the limitations of flow rate and mobile phase compositions encountered with electrospray and thermospray. This paper focuses primarily on chemical ionization applications sometimes utilizing a heated target to assist in the formation of molecular ions from involatile biological conjugates such as estrone glucuronide, estrone sulfate, androsterone glucuronide, 8-hydroxyquinoline glucuronide, and s-(N-methylcarbamoyl) glutathione.

The momentum separator and nebulizer were directly coupled to the 4 inch conflat flange on the left side of the Finnigan 4021. A bridge was constructed for chemical ionization which tees the CI gas directly after the momentum separator. There were no other modifications to the basic mass spectrometer making this a very easy retrofit. The Finnigan ion volume design allows for a DCI probe to be inserted through the front probe lock. This proved to be an important feature since we found it necessary to have a heated target in order to see molecular ions from some of the conjugates.

Using caffeine as the test compound it was determined that it was possible to obtain a reasonable spectra from 500 pg flow injected with 100 % methanol as the mobile phase flowing at 0.5 mL per min operating in the electron impact mode. This represents roughly a factor of 10 increase in sensitivity over any commercially available system.

Figures 1 and 2 are PCI ammonia spectra of s-(N-methylcarbamoyl) glutathione (SMG) with the heated target off, then on respectively. Notice the total absence of the pseudomolecular ion at m/z 365 in the target off spectrum. The presence of the heated target produces the pseudomolecular ion as a major ion. A comparison was made between data obtained via heated target particle beam and direct probe LSIMS of SMG. It was found that while the pseudomolecular ion was produced via LSIMS, it was necessary to perform MS/MS to obtain the same degree of structural information that was present in the PCI data.

The various conjugates were attempted via either positive or negative chemical ionization (ammonia moderating gas). The mobile phase was 50/50 water-methanol flowing at 0.5 mL per min. Optimization of the interface temperatures was accomplished first with caffeine, then with the individual compounds of interest to determine the best heated target temperature (if it was necessary). The actual temperature of the heated target is not known, only the voltage and current required to see the molecular ion of the more difficult species. Typically, these values were 4 volts drawing 0.1 amps of current. It was found that not all of the conjugates required the heated target to see the molecular ion.
Particle beam LC/MS is just beginning to reveal that it has applications for thermally labile compounds that in the past would have to be derivatized and run via a static probe technique such as LSIMS or FD. In fact, work has been done coupling particle beam with both LSIMS$^1$ and FD$^2$. The current developments in electrospray also show promise in analyzing compounds of similar nature with greater sensitivity, however particle beam unlike electrospray is capable of performing on-line analysis at "real world" flow rates making it possible to match LC conditions without the need for flow splitting. Therefore, enhanced sensitivity of electrospray may well indeed be offset by the need to split flow in order to achieve sample introduction rates in the microliters per minute range. For example if one were to split 0.5 mL/min to 1 uL/min, the resultant loss in sample delivered to the mass spectrometer (e.g. 1/500) would translate detection limits via electrospray in the 500 pg range to 250 ng via particle beam. Some of the data presented herein was collected at or lower than this figure.

Although micro LC is easily coupled with electrospray the sample capacity is limited and is virtually of no practical use when working with crude biological matrices such as urine.

References


2. W. Ligon, private communication
Particle Beam/MS Analysis of Naturally Occurring Aromatic Acids and Phenols


Aromatic acids and phenols, such as vanillyl and syringic acid, occur naturally in water and provide the building blocks for the lignins produced by vascular plants. As such, they are excellent molecular markers for natural water with a terrestrial source component. By analyzing the CuO oxidation products of lignins and dissolved humic materials, the macromolecular material has been correlated to specific types of vascular plants, i.e. gymnosperms versus angiosperms (e.g. Hedges and Ertel 1982, Ertel et al. 1986). CuO oxidation produces the single-ring aromatic acid and phenolic compounds mentioned above. Typically, these compounds are derivitized prior to analysis to form the more volatile alkylated silyl or methyl ethers which facilitates analysis by gas chromatography. However, silylation produces unstable derivatives and introduces appreciable amounts of contamination and methylation of the acidic and phenolic oxygens with diazomethane involves a mutagenic precursor. Furthermore, derivatization is time consuming with inherent losses of analytes due to the additional analytical step. Thus, direct analysis of these analytes, such as by HPLC/particle beam/MS, would eliminate the added analytical workup as well as the drawbacks described above.

Spectra of a series of substituted aromatic acids and phenols are presented here as introduced by the particle beam (PB) versus the gas chromatographic (GC) interface. Compounds intended for gas chromatographic introduction were derivitized by diazomethane prior to injection. The compounds analyzed were as follows: (a) phenolic methoxylated benzaldehydes vanillyl, syringaldehyde, and ethyl vanillyl (utility as a surrogate for recovery purposes); (b) phenolic methoxylated benzoic acids vanillic acid, syringic acid, p-coumaric acid, and ferulic acid; phenolic methoxylated acetophenones acetovanillone and acetosyringone; and (d) vanillyl alcohol. Vanillyl alcohol was not completely derivatized; thus, the GC spectrum is shown for the non-derivatized form. Sensitivity was roughly 10 to 100 times less for PB introduced compounds.

All spectra are well characterized by two or three fairly intense ions, including the molecular ion. Vanillyl alcohol is the only compound for which a direct comparison (both underivatized) may be made of PB versus GC effect on fragmentation patterns. The molecular ion is much larger in the PB spectra with a neutral loss of hydroxy apparent versus a loss of water for the GC spectra. Mid-range fragments (C7H7O+ m/z 107, and C6H5O+, m/z 93) are present in both spectra, although at different intensities. The aldehydes, except ethyl vanillyl, exhibit base peaks of either the M+ or (M-H)+ ion, with significant losses of CHO and CH3. Ethyl vanillyl, on the other hand, is dominated by the loss of 29, which may be a combined loss of CHO and C2H5. Loss of 28, most likely CO, was also noted for both its PB and GC spectra. PB and GC spectra for acetovanillone and acetosyringone were virtually identical in character, with base peaks attributed to the loss of CH3, molecular ions ranging from 38 to 57%, and loss of the acetyl group accounting for ions ranging in intensity of 10 to 31%. In both PB and GC spectra for the four acids, molecular ions dominated, with the exception of the GC spectra of p-Coumaric acid where loss of the methoxy group dominated. Losses of hydroxyl or methoxyl groups and COOH and COOCH3 were the mode for the PB and GC spectra, respectively.

References
TABLE I: PREDOMINANT IONS (Particle Beam-underivatized; Gas Chromatographic-methylated, *except vanillyl alcohol.  
All ions positive, electron energy 70eV, source T-250°C.)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Pred. Ions (Relative Intensity, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mode</td>
</tr>
<tr>
<td>Vanillyl</td>
<td>PB</td>
</tr>
<tr>
<td></td>
<td>GC</td>
</tr>
<tr>
<td>Syringaldehyde</td>
<td>PB</td>
</tr>
<tr>
<td></td>
<td>GC</td>
</tr>
<tr>
<td>Acetovanillone</td>
<td>PB</td>
</tr>
<tr>
<td></td>
<td>GC</td>
</tr>
<tr>
<td>Acetosyringone</td>
<td>PB</td>
</tr>
<tr>
<td></td>
<td>GC</td>
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<td>Vanillic acid</td>
<td>PB</td>
</tr>
<tr>
<td></td>
<td>GC</td>
</tr>
<tr>
<td>Syringes acid</td>
<td>PB</td>
</tr>
<tr>
<td></td>
<td>GC</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>PB</td>
</tr>
<tr>
<td></td>
<td>GC</td>
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<tr>
<td>Ferulic acid</td>
<td>PB</td>
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<tr>
<td>Vanillyl alcohol</td>
<td>PB</td>
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<tr>
<td></td>
<td>GC</td>
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</tbody>
</table>
Comparison of Thermospray and Particle Beam LC-MS for the Analysis of Triglycerides in Cooking Oils

Yang, Y-P and Vestal, Christina H.*. Vestec Corporation, Houston, TX

INTRODUCTION

Earlier work (1,2) has shown that Thermospray™ LC-MS is an effective tool for on-line identification of triglycerides separated by reversed-phase HPLC. Despite this success there remain some problems for which the information provided by Thermospray is not sufficient. Triglycerides with no carbon-carbon double bond often do not produce an observable protonated molecular ion using the Thermospray filament-on mode of operation required by the organic mobile phase. Those containing at least one C=C double bond always yield some protonated molecular ions and simple fragmentations which allow the masses of the three fatty acid components to be unambiguously determined. More recently, we have applied Particle Beam LC-MS to this problem and an improved LC method for Thermospray analysis to obtain detectable molecular ions for both the saturated and unsaturated triglycerides as well as structurally significant fragmentation. The resulting LC-MS techniques have been applied to analysis of a variety of commercial cooking oils to both identify and quantify the triglycerides present.

EXPERIMENTAL

Liquid Chromatography/Mass Spectrometry was performed using a Waters 600MS Multisolvent delivery system coupled to a Vestec 201 Dedicated Thermospray-Particle Beam Mass Spectrometer with an on-line UV detector at 215nm and connected to a Compaq 386 computer via a Teknivent Vector One interface. The LC column was an Alltech Spherisorb ODS-2, 4.6mm x 25cm, and 5 micron particle size. Samples were run isocratically using 52/30/18 acetonitrile/isopropanol (with 0.005M ammonium acetate)/hexane. Flow rate was 1.0 mL/min. All of the cooking oils were dissolved (50/50 v/v) in hexane prior to injection. The injection volume was 10 microliters. In this work we ran the following commercial cooking oils: "Mazola" Corn Oil, "Kroger" Vegetable Oil, "Wesson" Sunflower Oil, "Heart Beat" Canola Oil, and "Pompeian" Olive Oil which were labeled "no cholesterol" and "low saturated fat present" by the manufacturers. The experiments were done using Thermospray (TS) positive ion and filament-on mode of ionization and Particle Beam (PB)-EI at 20 eV.

RESULTS AND DISCUSSION

Figure 1 shows the TS (a) and the PB-EI (b) mass spectra of the standard tripalmitin (PPP) which is a completely saturated triglyceride. Both of the spectra gave a base peak at m/z 551 (PP⁺) and 39% of ammonium adduct at m/z 824 of PPP in TS and 1% of molecular ion M⁺ at m/z 806 in PB-EI. Saturated triglycerides do not produce observable molecular ions without some ammonium acetate buffer present in the mobile phase, and it also enhances the sensitivity without losing the chromatographic performance. The PB-EI spectrum at 20 eV of tripalmitin also consisted of several strong peaks at m/z 239 and 313 which are related to palmitic acid and monopalmitin with a loss of an OH group or water. The common fatty acids of triglycerides present in the cooking oils mentioned above are listed in Table I. Figure 2 shows the PB-EI total ion chromatogram (a) and the on-line UV trace at 215nm (b) for the analysis of "Heart Beat" Canola Oil. The TS- and PB-EI spectra of the peak at 7.7 minutes are shown in Figure 3 which consisted of common peaks at m/z 597 (LL⁺), 599 (OL⁺), and 601 (OL⁺) and other peaks at m/z 95, 108, 260, 262, 337, and 878 for PB-EI and 879 and 896 for TS. It determines the molecular weight of 878 daltons and three fatty acid components of oleic (O), linoleic (L), and linolenic (U) of this triglyceride which is present in "Heart Beat" Canola Oil. Four other cooking oils also show a peak at around 7.5 minutes with MW 878, but only have a single ion at m/z 599. It indicates the presence of trilinolein (LLL) in these cooking oils. It was also found a small amount of triglycerides of behenic acid (C22H43COOH) present in "Wesson" Sunflower Oil. The TS spectrum at 16.3 minutes of this triglyceride is shown in Figure 4 which consisted of a base peak at m/z 939 (MH⁺) and intense peaks at m/z 899 (LL⁺) and 659 (LB⁺). Unfortunately, it did not give a good PB-EI spectrum.

CONCLUSIONS

Thermospray and Particle Beam LC-MS with buffer present in the mobile phase is a powerful tool for on-line confirmation and identification of triglycerides separated by reversed-phase HPLC. The amount of unsaturated and saturated triglycerides present in cooking oil agrees with the manufacturers' values shown in Table II.

REFERENCES


Table I. Common Fatty Acids of Triglycerides Present in Cooking Oils

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>MW</th>
<th>Unassigned</th>
<th>Assigned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermospray</td>
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<tr>
<td>Palmitic</td>
<td>236</td>
<td>Palmitic</td>
<td>C15H30COOH</td>
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<tr>
<td>Linoleic</td>
<td>264</td>
<td>Linoleic</td>
<td>C17H30COOH</td>
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<tr>
<td>Arachidonic</td>
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<td>Arachidonic</td>
<td>C18H32COOH</td>
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<tr>
<td>Serrinic</td>
<td>340</td>
<td>Serrinic</td>
<td>C19H34COOH</td>
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Table II. Brand Name and Saturation Analysis

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>%OH</th>
<th>%Double Bond</th>
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<tbody>
<tr>
<td>Heart Beat Canola Oil</td>
<td>5.6</td>
<td>1.4</td>
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<tr>
<td>Mazola Corn Oil</td>
<td>17.0</td>
<td>4.2</td>
</tr>
<tr>
<td>Kroger Sunflower Oil</td>
<td>19.0</td>
<td>4.2</td>
</tr>
<tr>
<td>Pompeian Olive Oil</td>
<td>17.0</td>
<td>17.0</td>
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</table>
THERMOSPRAY AND PARTICLE BEAM LC-MS, COMPARATIVE STUDY FOR SOME SESQUITERPENE LACTONES FROM ACROPTILON REPENS.

Leslie A. Harden, Kenneth L. Stevens, and William F. Haddon, Western Regional Research Center, USDA 800 Buchanan St., Albany, CA. 94706.

Introduction. Sesquiterpene lactones occurring in Russian knapweed (Acroptilon repens L.) have been implicated in the nervous system disorder in horses known as equine nigropallidal encephalomalacia. Identification of these natural products from complex mixtures has in the past been aided by use of the mass spectrometer only after their isolation as pure compounds.

With the advent of practical LC/MS interfaces, the mass spectrometer can provide information to the natural products chemist that allows target compound analysis and structure elucidation of unknowns in complex mixtures. In this study we targeted the compounds previously isolated from Russian knapweed, repin, acroptilin, and centaurepensin. We compared particle beam (P/B) El, positive and negative ion Cl, as well as positive and negative ion thermospray (TSP) spectra for these sesquiterpene lactones and applied this information to the analysis of a plant extract from Russian Knapweed.

Experimental. All analyses were carried out with an Extrel C-50/400 quadrupole mass spectrometer equipped with TSP and P/B supplied by that manufacturer. The sample introduction and chromatography were performed on a Waters 600E HPLC equipped with an Analytichem C-18, 4mm x 15cm, column with 4.5 um packing.

Initial evaluation P/B (El, methane Cl, and ammonia Cl) and TSP were performed by sample introduction of individual compounds via flow injection without chromatography. The mobile phase in the P/B experiments was acetonitrile at 0.6mL/min. System temperatures for Cl in degrees centigrade were: P/B desolvation chamber=83, P/B control=125, P/B tip=192, MS source=210. For the TSP experiments 29% acetonitrile, 71% 0.1 M ammonium acetate in water was used at 1.2mL/min. Relevant temperatures in degrees centigrade were: source block=207, TSP probe control=127, and TSP probe tip=204.

Chromatographic TSP studies were performed on a mixture of repin, acroptilin, and centaurepensin as well as an extract from Acroptilon repens using an isocratic mobile phase of 29% acetonitrile, 71% 0.1M ammonium acetate in water.

Results. Of the three target analytes, the sensitivity was least for centaurepensin. Detection in the P/B mode was achieved at 1ug with negative ion ammonia Cl (Figures 1-3). Negative, molecular ion, ammonium adducts were seen down to 250ng for this compound when flow injected in the TSP mode (Figures 4-6).

Molecular ion abundances and sensitivity for the target analytes appeared to be enhanced with negative ion detection versus positive ion detection for both P/B, with Cl, and TSP ionization. Analysis of the knapweed extract, illustrated by single ion current plots (Figures 7 and 8), reveals an apparent mixture of components at the location of centaurepensin elution in the chromatography scheme utilized.

Discussion. Comparison of the spectra derived in the LC-MS analyses of the known compounds and the plant extract in this study demonstrate the utility of this hyphenated technique in providing useful information to the natural products chemist. In this case revealing a mixture of components that would not be recognized without additional separation. In addition this technique promises to provide additional information on the character of unidentified constituents in complex mixtures.

1. P/B o«0a£l«« loo UMOI CI «p*ctru» for c«ntmr*p«Baia

Figure 1. P/S negative ion ammonia CI spectrum for repisa.

Figure 2. P/S negative ion ammonia CI spectrum for acroptilin

Figure 3. P/S negative ion ammonia CI spectrum for centaurapensin

Figure 4. Negative ion TSP spectrum for repisa

Figure 5. Negative Ion TSP spectrum for acroptilin

Figure 6. Negative Ion TSP spectrum for centaurapensin

Figure 7. TIC and selected single ion profiles for target compounds chromatographed on C-18 RPIC column.

Figure 8. TIC and selected single ion profiles for Russian knapped extract chromatographed on C-18 RPIC column.
APPLICATION OF LC/MS TO THE STUDY OF CHELATORS IN MIXED HAZARDOUS WASTES

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Chelators, such as ethylenediaminetetraacetic acid (EDTA), citric acid, and N-(2-hydroxyethyl)ethylenediaminetriacetic acid (HEDTA) have been used to complex radionuclides in mixed hazardous wastes (MHW). These organoradionuclides are potentially more environmentally hazardous than either radionuclides or chelators alone due to their enhanced mobility through the soil and possible deposition into the ground water. In addition, many of the chelators can't be analyzed by either gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS), accepted EPA techniques, without first being derivatized. Derivatization, however, may require extra sample handling, may give low or variable recoveries, and often requires the use of hazardous reagents. As a result, we have evaluated liquid chromatography/mass spectrometry (LC/MS) utilizing the HP 5988A LC/MS as a potential technique for analyzing chelators and chelator fragments. We have examined both the free acids and Cu complexes of several chelators and chelator fragments by LC/MS.

Both thermospray and particle-beam LC/MS interfaces are available in our laboratory. The chromatographic system for both particle-beam and thermospray was a HP Model 1090 equipped with a ternary solvent delivery system, a variable volume sample injector, an auto sampler, and a column bypass valve. The HP Model 5988A particle-beam and thermospray interfaces and mass spectrometer were used in this study. For the thermospray studies, the source temperature was typically 300° C and the stem temperature was 90° C. The mobile phase was 0.1M ammonium acetate. The free acids (not complexed) were analyzed using flow injection techniques. Separation of the copper complexes of EDTA and HEDTA was accomplished using RP2 Lichrosorb column with ammonium acetate as the buffer at a flow rate of 1 ml/min. Several chelators were also analyzed by direct injection techniques in methanol and particle-beam LC/MS.

Figure 1a is the thermospray LC/MS mass spectrum of HEDTA (MW 278). The base peak is m/z 261. This is probably the result of protonation to form m/z 279 and then loss of water to form m/z 261. The ion m/z 261 could also be the protonated lactam, but then one would expect an addition of 18 as the ammonium adduct at m/z 278. Figure 1b is the mass spectrum of ethylenediaminetriacetic acid (ED3A), a decomposition product of HEDTA. The ion m/z 217 is probably the protonated lactone and 234 is (M+18). The formation of lactones and lactams was also noted by Toste (1) in the GC/MS analysis of methylated derivatives of several of the chelators.

The mass spectra of various chelators obtained by particle-beam LC/MS showed extensive fragmentation; the chelators could not be unambiguously identified based on the spectra.

A LC method for the separation of chelators and chelator fragments utilizing Cu complexation, reverse phase, ion-pair chromatography has been developed by W.R. Grace and modified by our group. As a result, we have examined several Cu complexed chelators by LC/MS. Figure 2a is the total ion chromatogram of a mixture of Cu(EDTA) and Cu(HEDTA). Figure 2b and 2c are mass spectra of the complexed chelators. It is interesting to note that the mass spectrum of Cu(HEDTA) is similar to the mass spectrum of ED3A. Cu may be facilitating electron transfer and subsequent reduction. Further studies are underway to determine the role of copper.

Thermospray LC/MS can be utilized to identify the chelators and the complexed chelators and chelator fragments. Particle-beam LC/MS, however, may have limited application, due to extensive fragmentation.
References


** FIGURE 1. (a) The Thermospray LC/MS spectrum of HEDTA and (b) EDTA.**

** FIGURE 2. (a) Total Ion Chromatogram of Cu(EDTA) and Cu(HEDTA), (b) Thermospray LC/MS mass spectrum of Cu(EDTA) and (c) Cu(HEDTA).**

** This work was supported by the Department of Energy under contract DE-AC06-76RLO 1830. Pacific Northwest Laboratory is operated by Battelle Memorial Institute.**
Accurate Mass Determinations By Thermospray LC/MS

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Boehringer Ingelheim Pharmaceuticals Inc., R & D Center, Ridgefield, CT 06877

Accurate mass determination in conjunction with thermospray ionization has been investigated. Modifications to instrument hardware and improved methods of calibration permit us to obtain resolution and stability comparable to standard EI performance. Measurement accuracy of better than 7 ppm for low level components separated by HPLC and better than 2 ppm for samples introduced directly into the mass spectrometer was achieved.

The instrument was fitted with an improved vaporizer insert, having a replaceable sapphire orifice, which greatly improved ion beam stability. The mass spectrometer was also modified by the addition of a cryopump to the source housing. This lowered both source and analyzer pressures which greatly enhanced resolution and sensitivity (Table 1). Large systematic errors in mass assignment were observed when operating with current controlled magnetic scanning. These errors were greatly reduced by operation with field controlled magnetic scanning (Fig. 1).

The remaining systematic errors (in field control) were further characterized. It was most convenient to perform this experiment using perfluorokerosene and EI ionization. A modified reference file of ions separated by 40-50 amu was constructed to simulate the use of polyethylene glycol oligomers (separation of 44 amu) as internal reference ions in TSP operation. The remaining PFK ions were treated as unknowns by the data analysis software. Two hundred scans were averaged to largely eliminate random statistical errors. The remaining systematic errors were used to derive a mathematical correction formula:

$$M_{corr} = M_{meas} + \left(0.038 \frac{|M_{meas} - M_{ref}|}{M_{meas}}\right)$$

where $M_{corr}$ is the calculated exact mass of the unknown, $M_{meas}$ is mass of the unknown reported by the instrument (DS90) software, and $M_{ref}$ is the mass of the nearest internal reference ion. This formula was found to be accurate over the mass range 500-1000 and independent of scan rate.

Several test compounds were analyzed and the correction formula was applied to the average of about 30 scans. As shown in Table 2, excellent results were obtained.

A Kratos MS25RFA mass spectrometer equipped with a thermospray interface was employed for these experiments. The LC system consisted of an ABI SF400 pump and SF757 UV detector having a high pressure in-line flowcell. An additional SF400 pump was connected via a low dead volume tee to permit post-column addition of internal standard calibrant. The standard EI source was used for the PFK experiments. A Vacumetrics 33060 LN$_2$ cryopump was added to the ion source housing by removing the glass plate and adding an aluminum adapter plate constructed in-house. A Vestec VT146014R vaporizer tip having a replaceable sapphire orifice was installed. This required use of an insulator over the entire enclosed length of the insert and wrapping of its thermocouple wires to prevent high voltage discharge.

Test compounds were injected into a 0.1 ml/min flow of 50% acetonitrile in water with 0.015% PEG 200-400 (1:1). Make-up flow was 1.0 ml/min of 0.3 M ammonium acetate. A WISP auto injector was employed to inject 0.5 ml of sample solution containing 100 ug of test compound. This was sufficient to produce a strong signal for several minutes. Approximately 30 scans were averaged for each analysis.
The mass spectrometer was operated at 10 000 resolution (10% valley) with a scan rate of 10 sec/decade. The instrument was scanned from m/z 600 to 120 for the test compounds and 400 to 120 for the LC/MS experiment. Field controlled magnetic scanning was performed, except as noted.

### Table 1
Effect of Source Cryopumping

<table>
<thead>
<tr>
<th>Resolution</th>
<th>None</th>
<th>Cryopumped</th>
<th>El (Spec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st slit</td>
<td>580</td>
<td>720</td>
<td>700(600)</td>
</tr>
<tr>
<td>3rd slit</td>
<td>2 750</td>
<td>3 350</td>
<td>3 500 (3 500)</td>
</tr>
<tr>
<td>4th slit</td>
<td>4 000</td>
<td>9 000</td>
<td>10 000 (7 500)</td>
</tr>
<tr>
<td>Sensitivity at m/z 59</td>
<td>3.5V</td>
<td>7.0V</td>
<td></td>
</tr>
<tr>
<td>Source pressure</td>
<td>&gt;1.5 x 10^-4</td>
<td>3.0 x 10^-4 (torr)</td>
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</tr>
<tr>
<td>Analyzer pressure</td>
<td>1.0 x 10^-4</td>
<td>3.0 x 10^-4</td>
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### Table 2
Test Compound Results For TSP Accurate Mass

<table>
<thead>
<tr>
<th>Compound</th>
<th>Error Before Correction</th>
<th>Error After Correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-naphthylalane (399)</td>
<td>-2.75 ppm</td>
<td>-0.19 ppm</td>
</tr>
<tr>
<td>Adenosine 191 (268)</td>
<td>-5.53 ppm</td>
<td>-0.53 ppm</td>
</tr>
<tr>
<td>Mexiletine (180)</td>
<td>-14.43 ppm</td>
<td>-0.72 ppm</td>
</tr>
<tr>
<td>Bisacodyl (362)</td>
<td>-4.93 ppm</td>
<td>-0.15 ppm</td>
</tr>
<tr>
<td>BIRG 587 (257)</td>
<td>-5.97 ppm</td>
<td>-0.26 ppm</td>
</tr>
<tr>
<td>Chlorothalidone (321)</td>
<td>-6.99 ppm</td>
<td>0.56 ppm</td>
</tr>
<tr>
<td>Persantine (505)</td>
<td>-0.45 ppm</td>
<td>1.72 ppm</td>
</tr>
<tr>
<td>Adenosine 10/90</td>
<td>-5.65 ppm</td>
<td>0.53 ppm</td>
</tr>
<tr>
<td>C11 PEG (287)</td>
<td>-0.05 ppm</td>
<td>0.51 ppm</td>
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</table>

### Table 3
Results of Accurate Mass Measurement from LC/MS Analysis of Low Level Impurities in BIRG 587

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Proposed Formula</th>
<th>Determined Mass</th>
<th>Error (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C22H22N6O11</td>
<td>527.9965</td>
<td>-0.7 ppm</td>
</tr>
<tr>
<td>2</td>
<td>C22H22N6O11</td>
<td>527.9965</td>
<td>-0.7 ppm</td>
</tr>
<tr>
<td>3</td>
<td>C22H22N6O11</td>
<td>527.9965</td>
<td>-0.7 ppm</td>
</tr>
<tr>
<td>4</td>
<td>C22H22N6O11</td>
<td>527.9965</td>
<td>-0.7 ppm</td>
</tr>
<tr>
<td>5</td>
<td>C22H22N6O11</td>
<td>527.9965</td>
<td>-0.7 ppm</td>
</tr>
<tr>
<td>6</td>
<td>C22H22N6O11</td>
<td>527.9965</td>
<td>-0.7 ppm</td>
</tr>
<tr>
<td>7</td>
<td>C22H22N6O11</td>
<td>527.9965</td>
<td>-0.7 ppm</td>
</tr>
</tbody>
</table>

### Figure 1
Mass Measurement Accuracy Field vs. Current Control
Adenosine MH+ = 258.1046 at 9000 resolution

Reference:
SENSITIVE DETECTION OF DIHYDROXY ARACHIDONIC ACIDS FROM HUMAN POLYMORPHONUCLEAR LEUKOCYTES (PMNLs) BY THERMOSPRAY LC/MS

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Human PMNLs are known to metabolize arachidonic acid to leukotrienes and 5(S),15(S)-, 8(S),15(S)- and 5(S),12(S)-dihydroxyeicosatetraenoic acid (diHETE) by various lipoxygenase reactions. Leukotriene B₄ (LTB₄), one of the main dihydroxy compounds produced by PMNLs, induces chemokinesis, chemotaxis, aggregation and degranulation of human PMNLs and has been implicated as a potential mediator of allergy and inflammation. While 8,15-diHETE also exerts a chemotactic effect upon PMNLs, 5,12-diHETE antagonizes some of the LTB₄ effects. Measurements of these compounds are typically made using an HPLC/UV technique. However, this technique is not adequate for the analysis of many biological samples, since its detection limit is usually above the ng range and these compounds are generally present only in nanomolar concentrations.

In our laboratory, these hydroxy compounds were measured by thermospray LC/MS after pentafluorobenzyl (PFB) derivatization. As demonstrated in Figure 1, subnanogram quantities of PFB derivatives of leukotriene B₄ and various diHETE standards can be easily detected. Approximately 250 pg of diHETEs were separated using reverse phase HPLC (C-18, 2.1 mm x 10 cm, 5µ) with a mobile phase of 0.1M ammonium acetate/acetonitrile (40/60) and a flow rate of 1 mL/min. They were then detected in an on-line fashion by a negative ion mode-thermospray technique in either a discharge-on or filament-on condition. The source and vaporizer tip control temperatures were maintained at 250 and 75°C, respectively. With this technique, 50-100 pg of dihydroxy compounds can be quantified after HPLC separation.

Using ¹³C₂-LTB₄ as an internal standard, the production of leukotriene B₄, as well as other diHETEs, was measured from PMNL preparations. Whole blood was obtained from normal volunteers and PMNLs were separated using a Histopaque density gradient method. Recovered PMNLs were washed with phosphate buffered saline and resuspended in the same buffer. Their capacity to produce these hydroxy compounds was determined after stimulation with the calcium ionophore A23187. The metabolites were extracted with ethylacetate in the presence of 2 ng of ¹³C₂-LTB₄ as an internal standard. The result obtained from 1x10⁶ purified PMNLs (corresponding to approximately 5 mL of whole blood) is shown in Figure 2. For this particular experiment, in order to better separate 8,15- and 5,15-diHETE, an ODS column with 3µ particle size (4.6 mm x 7.5 cm) was employed with the same mobile phase described above. The PMNLs were observed to produce LTB₄ and 8,15-, 5,15- and 5,12-diHETE, and the levels of these compounds varied between individual blood samples. This was presumably due to individual variation in the content of precursor arachidonic acid as well as in the sensitivity of the PMNLs to stimulation. However, production of approximately 1-5 ng of these compounds was estimated with reference to the internal standard. Typically, this level of products could not be analyzed by UV detection either before or after derivatization.

This technique was employed in order to investigate the effect of ethanol on the production of these dihydroxy compounds. The purified PMNLs were preincubated with 0.01-0.2% ethanol for 10 min prior to the stimulation by A23187. The levels of 5,15-diHETE, LTB₄ and 5,12-diHETE were measured by thermospray LC/MS after PFB-derivatization. As shown in Table 1, no difference was found in their production. However, it is possible that changes in their production could be observed following a longer period of preincubation of PMNLs with ethanol.
Figure 1. Separation and detection of standard diHETEs (approximately 250 ng each).

Figure 2. Production of diHETEs by human PMNLs.

Table 1. Effect of ethanol on production of diHETEs by human PMNLs.

<table>
<thead>
<tr>
<th>Ethanol (v/v%)</th>
<th>5,15-diHETE</th>
<th>LTB4</th>
<th>5,12-diHETE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.2</td>
<td>4.6</td>
<td>3.1</td>
</tr>
<tr>
<td>0.01</td>
<td>1.2</td>
<td>4.6</td>
<td>4.0</td>
</tr>
<tr>
<td>0.1</td>
<td>1.1</td>
<td>4.0</td>
<td>3.0</td>
</tr>
<tr>
<td>0.2</td>
<td>1.1</td>
<td>4.3</td>
<td>3.0</td>
</tr>
</tbody>
</table>
THERMOSPRAY LC/MS ANALYSIS FOR CONTAMINANTS IN L-TRYPTOPHAN SUPPLEMENTS

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At the end of 1989 a new fatal disease called eosinophilia-myalgia syndrome (EMS) appeared in America and West Germany. Epidemiological investigations traced the cause to an unknown contaminant in L-tryptophan (LT) dietary supplement tablets (1-3). High performance liquid chromatography (HPLC) analysis with UV detection found more than 50 contaminants in the LT material. Many of the contaminants were present in less than parts per million levels.

One approach to identifying these contaminants was analysis with thermospray LC-MS or LC-MS/MS. These analyses were performed with the Finnigan TSQ-70 triple quadrupole mass spectrometer with the thermospray interface and the Waters 600 liquid chromatography system with an online Waters 490 UV detector. Elution was by a water and acetonitrile linear gradient that contained 0.1% trifluoroacetic acid (TFA). Both discharge ionization at 0.5 kV and buffer ionization were used. The thermospray vaporizer was set at 120 C and the block was set at 240 C. For MS/MS analyses, the collision gas was argon; the collision cell pressure was 1.3 millitorr; and the collision offset was -10 V.

Thermospray was useful in attempting to identify some of the more concentrated contaminants but was not sensitive enough to detect many of the lower concentration contaminants. The four most prominent UV detectable contaminants present in LT tablets at concentrations between 10 and 200 ppm were easily detected and LC-MS/MS spectra were obtained. These compounds are varieties of indole-tryptophan conjugates with molecular weights between 300 and 400 amu; it is unlikely that they are EMS causative agents since they are present in all the LT lots including those not associated with EMS cases. Thermospray was useful in identifying the LT contaminant 1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid (MCCA), which was not case related but was of interest because it is a breakdown product of a compound that was case related (see below). Thermospray was used to identify two other contaminants, 5-chloro-LT and 5-hydroxy-LT, neither were EMS case related.

Of the many contaminants found in the LT preparations, the only one that had a marked association with EMS cases was identified as 1,1'-ethylidenbis[L-tryptophan] (ETB), also known as "peak 97" and "peak E" (4,5). EBT is formed during the LT manufacturing process probably by the reaction of 2 LT molecules with an acetaldehyde molecule. Because EBT is thermolabile, breaking down in the thermospray ion source mainly to LT and a small amount of MCCA, thermospray was only of limited use in identifying EBT. Atmospheric pressure ionization (API) LC-MS analysis provided much more useful information in the identification of EBT because the 435 m/z MH+ ion was prominent in
the EBT spectrum. API LC-MS was between 10 and 100 times more sensitive for many of the LT contaminants than thermospray.

Bacitracin, a 1420 amu antibiotic bacterial peptide that under certain culture conditions is synthesized by the bacterial strain used in the production of LT, is a suspected LT contaminant. The thermospray limit of detection for bacitracin was about 5 ppm in spiked LT samples using a narrow scan window and a 20 kV dynode detector. We were not able to detect bacitracin in the LT material by thermospray.

Since no extensive sample preparation was done before LC-MS analysis, a very large amount of LT entered the ion source each run. Thermospray proved to be very rugged and reliable under these conditions, requiring only weekly ion source cleaning.

REFERENCES

INTRODUCTION

Sulfonamides are an important class of antibacterial agents used in veterinary practice. Sulfonamides are fed to cattle for prophylactic and therapeutic purposes. Residues from sulfonamides have been found in cows' milk as well as edible animal tissues. A recent study implies that sulfamethazine may be a thyroid carcinogen.¹ Various analytical methods have been developed for the analysis of sulfonamide residues in milk.²-⁴ Liquid chromatography/mass spectrometry has been utilized for the determination of sulfadiazine in biological fluids and for the analysis of sulfonamide residues in meat.⁵,⁶ Combined high-performance liquid chromatography/thermospray mass spectrometry (HPLC/TSMS) is used in this study for the analysis of sulfamethazine (SMZ), sulfamerazine (SMR) and sulfadiazine (SDZ).

EXPERIMENTAL

An ISCO LC-5000 syringe pump was connected to a Rheodyne 7125 injector equipped with a 20 µl loop. A Phase-Sep Spherisorb S5 ODS2 (150 x 4.6 mm) column, preceded by a Brownlee RP-18 New Guard column (15 x 3.2 mm), connected the injector directly to a Delsi Nermag RIO-10C mass spectrometer. The mobile phase was 0.1 M ammonium formate in an 80% water/20% acetonitrile mixture pumped at 1.1 ml/min. The mass spectral analyses were performed in the positive ion, "filament/discharge off" and "repeller off" mode. The control temperature and the block temperature were set at 130°C and 150°C, respectively. Full scans of m/z 125 - m/z 300 were acquired for the standard solution and scans of m/z 225 - m/z 300 were acquired for the blank and fortified milk samples.

Standard solutions of SMZ, SMR and SDZ (ICN Pharmaceuticals) were prepared at a concentration of 1.0 mg/ml in mobile phase. Milk was fortified by the addition of 100 µl each of the standard solutions to 10 ml of milk to yield a final concentration of 10 ppm. The milk used was whole milk purchased locally. Both the blank milk and the fortified milk samples were mixed 50/50 with mobile phase before injection.

RESULTS AND DISCUSSION

The structures of the three sulfonamides are shown in Figure 1. Good separation was obtained when a 5 ppm mixture of the three standards was analyzed by HPLC/TSMS. The base peaks in the TS mass spectra of the three standards corresponded to the [MH]+ ions for the individual compounds.

No evidence of sulfonamide residues was present in an unspiked milk sample analyzed by HPLC/TSMS. There was a strong early signal due to the milk components. Figure 2 displays the HPLC mass chromatogram and ion traces for a 10 ppm fortified milk sample. The ion traces represent the [MH]+ ions of each compound. Good resolution is still achieved for the three standards along with a well resolved early peak due to the milk matrix.

CONCLUSION

The utility of HPLC/TSMS for the quick analysis of sulfamethazine and two other sulfonamides in milk has been demonstrated. The technique may also prove useful for analysis of other sulfonamides. Detection limits were not at optimum levels in this study. By experimenting with various extraction techniques, selected ion monitoring or selected reaction monitoring, the detection limits for sulfonamides by HPLC/TSMS should be improved significantly.

ACKNOWLEDGEMENTS

We would like to acknowledge Eugene B. Hansen, Jr. for his help in the liquid chromatography development and J. P. Freeman for his assistance with the Generic CADD program.
Figure 1 Structures of Sulfonamides

![Structures of Sulfonamides](image)

Figure 2 HPLC/TSMS of 10 ppm fortified milk mixture of SDZ, SMR and SMZ. 100 ng of each injected.

REFERENCES
UTILITY OF HPLC-TSMS FOR THE ANALYSIS OF THE ANTITUBERCULOSIS DRUGS ACONIAZIDE AND ISONIAZID

E.B. Hanson, Jr., J. Bloom, H.C. Thompson, Jr., and W.A. Korfmacher; Food and Drug Administration, National Center for Lexicological Research, Jefferson, AR 72079

BACKGROUND

Since 1952 the drug of choice for the treatment of tuberculosis (TB) in humans has been isoniazid (INH). Because INH can cause severe side effects there has been a search for less toxic drugs (1). Aconiazide (ACON), is the isonicotinylhydrazone of 2-formylphenoxyacetic acid. ACON has been reported to provide antituberculosis activity both in animals and humans equivalent to that of INH and has demonstrated a much lower toxicity (2). Presently, animal toxicological studies of ACON are being performed at the FDA's NCTR and analytical procedures for the analysis of ACON and INH in aqueous solution and rat plasma are needed to support these studies.

EXPERIMENTAL

ACON solutions were prepared at about 1 mg/ml by dissolving a weighed amount of the powder into an appropriate volume of 1% aqueous sodium bicarbonate (w/v).

Mass spectral analyses were performed using a Finnigan MAT TSQ 70 triple stage quadrupole mass spectrometer equipped with a Finnigan MAT thermospray controller and source. The mobile phase was maintained at a flow rate of 1.25 ml/min by an Isco LC-5000 syringe pump. Typically, the vaporizer was set to 105°C with a block (zero) setting of 220°C.

HPLC was achieved for ACON using a Beckman ODS analytical column. The mobile phase was 600 ml water and 400 ml methanol to which was added 5 grams of ammonium formate. For the analysis of INH in rat plasma a Beckman CN analytical column was used and the mobile phase was 650 ml of water and 350 ml methanol which also contained 5 grams of ammonium formate. Typically, 20 µl aliquots of sample were injected on column using a Rheodyne model 7125 injector.

Formation of the isonicotinylhydrazone of verataldehyde (3,4-dimethoxybenzaldehyde) in rat plasma was achieved using a modification of a previously reported procedure (3). A 250 µl aliquot of rat plasma spiked with 10 ppm INH was mixed with 50 µl of a 1.5% methanolic solution of verataldehyde and 100 µl of a 10% aqueous solution of trifluoroacetic acid with a vortex mixer for 1 min. The samples were then centrifuged at about 2000 rpm for 10 minutes and the supernatant analyzed by HPLC-TSMS.

RESULTS AND DISCUSSION

Figure 1 shows the results from the HPLC-TSMS analysis of a freshly prepared aqueous aconiazide solution. The large peak in the RIC in Figure 1 is due to ACON. The TS mass spectrum obtained for this peak showed an ion at m/z 300.
which is consistent with the \([M+H]^+\) ion for ACON; the spectrum showed a base peak at m/z 138 which can be attributed to fragmentation at the C-N bond of the ACON molecule.

Figure 2 shows the mass chromatograms from the analysis of an old aconiazide solution. The RIC in Figure 2 shows that the sample contains three major components; these HPLC peaks are labeled 1 to 3. Peak 1 is identified as ACON because its retention time and its TS mass spectrum match those of the standard material. Peak 2 is identified as 2-formylphenoxyacetic acid (2-FPAA) because its TS mass spectrum (consisting mainly of peaks at m/z 180 and m/z 198) and HPLC retention time match those of authentic 2-FPAA. The TS mass spectrum for HPLC peak 3 includes at least two components which are only partially resolved; one of the components is INH since the TS mass spectrum for peak 3 includes a strong peak at m/z 138, which is the \([M+H]^+\) ion for INH. In addition, this TS mass spectral response and HPLC retention time match those of authentic INH. The other component of peak 3 has not been identified, but the TS mass spectral data for this compound includes ions at m/z 142, m/z 156, and m/z 169.

In addition, there are at least two other minor components that were observed in the HPLC-TSMS analysis of the aged ACON solution shown in Figure 2. One of these components corresponds to the HPLC peak labeled 4, and has a TS mass spectrum consisting of a base peak at m/z 200 with no additional ions of significance. The other component is labeled 5 and has a TS mass spectrum which includes a base peak at m/z 227 with no other ions of significance.

The HPLC-TSMS mass chromatogram of a 1 mg/ml INH equivalent standard solution reacted to form the isonicotinylhydrazone of veratadehyde shows two major peaks eluting at 5.5 and 9 minutes and are attributed to excess veratadehyde and its isonicotinylhydrazone, respectively. The TS mass spectrum of the 5.5 minute peak showed a base peak at m/z 167 and was consistent with veratadehyde. The second peak seen at 9 minutes gave a TS mass spectrum showing essentially only the base peak at m/z 286 consistent with the isonicotinylhydrazone of veratadehyde.

Figure 3 shows the HPLC-TSMS analysis of a rat plasma sample which was spiked with 10 ppm INH, reacted to form the isonicotinylhydrazone, and analyzed by HPLC-TSMS. The peak seen at 9 minutes is identified as the isonicotinylhydrazone of veratadehyde because its retention time and the resulting mass spectrum obtained was identical with that obtained from the standard INH-veratadehyde analysis.

**CONCLUSIONS**

This study demonstrates HPLC-TSMS to be very useful for the analysis and characterization of the antituberculosis drugs aconiazide and isoniazid in aqueous solutions and in rat plasma samples.

**REFERENCES**

Diethylamine Cluster Ion Artifacts Observed During Thermospray LC/MS/MS
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Introduction: Diethylamine is often used as an additive in the mobile phases for reverse phase HPLC separation in order to enhance peak shape (reduce tailing) and adjust pH. The volatility of diethylamine makes it an ideal additive for thermospray liquid chromatography/mass spectrometry applications (LC/MS). Initial attempts to characterize the degradation products of nizatidine (Figure 1) by thermospray LC/MS utilized a mobile phase containing diethylamine that was successfully employed for the UV detection of the drug and its nitrous acid degradation products. The resulting thermospray spectra of the first eluting peak did not produce ions that could be interpreted as products of nizatidine. The spectra contained series of peaks separated by 171 mass units and all of the major ions had a molecular weight greater than the molecular weight of nizatidine. These peaks were analyzed by LC/MS/MS in a triple stage quadrupole mass spectrometer.

The daughter ion collisional induced dissociation (CID) spectra of each of the major ions in the first eluting peak produced a common daughter ion at m/z=74. An examination of the mobile phase components suggested that protonated diethylamine (M+H ion of 74 amu) might be involved. Subsequent reanalysis of the nizatidine reaction products with the same mobile phase minus the diethylamine component produced spectra that could be readily assignable as related to nizatidine. Apparently under the thermospray ion source conditions, diethylamine reacted with the components to form a series of cluster ions.

Methods: Reaction Conditions Nizatidine (2.5 g, 7.55 mmole) and sodium nitrite (2.1 g, 30.4 mmole) were added with stirring to water (760 ml) at 37 C in a 3-neck flask. Hydrochloric acid (20 ml of 1N) was added until pH 3.63 was achieved. The solution was stirred for 3 hours at 37°C. Ammonium sulfamate (4.33 g, 38.0 mmole) was added to consume the excess nitrous acid. The solution was lyophilized and stored at approximately -20°C. LC/MS Twenty five microliters (50 ug) of the sample dissolved in mobile phase was injected through a Rheodyne injector onto a 15 cm, 5 micron, Jones ODS reverse phase HPLC column. The mobile phase consisted of 760 ml of an 0.1 M ammonium acetate buffer (adjusted to pH 7.5 with glacial acetic acid) and 215 ml methanol. The diethylamine containing mobile phase had 1 ml of DEA added to the aforementioned mobile phase. The isocratic separation was achieved by pumping mobile phase at a flow rate of 1 ml/min through the column using a Waters 600-MS HPLC pump with Silk software. The HPLC eluent was delivered to a Nermag R 30-10 triple stage quadrupole mass spectrometer equipped with a Nermag thermospray ion source. The ion source block temperature was maintained at 223°C with a probe tip temperature of 206°C and a repeller voltage of 150 eV. LC/MS/MS The Thermospray ionization conditions were identical to the conditions listed above in the LC/MS method. A Nermag R 30-10 triple quadrupole mass spectrometer was operated with Q1 filtering the desired daughter ion. The second quadrupole, Q2, was set as an rf only collision cell that was maintained with a gas collision pressure of 2.5 x 10-2 torr Argon. The third quadrupole, Q3, scanned the collision induced dissociation fragments at a rate of 1 scan/second. The accelerating potential of the collision was increased by -40 eV to increase the internal energy of the isolated daughter in order to enhance the collision process.

Results and Discussion: The LC/MS analysis of nizatidine nitrosation reaction products using a diethylamine containing mobile phase produced four major peaks in the LC chromatogram (Figure 2). Spectra from the major peak revealed ions that did not seem related to the structure of nizatidine (Figure 3). These ions appeared in distinct groups in the spectra that were separated by 171 mass units. In the subsequent LC/MS/MS analyses, the daughter ion at 74 amu was consistently observed (Figure 4). Since no fragment of 74 mass units could be readily attributed to a nizatidine substructure the possibility of artifact formation was investigated. An examination of the mobile phase components suggested that protonated diethylamine was involved in the formation of the observed ions. Reanalysis of the nitrosation reaction products without diethylamine present in the mobile phase produced an LC/MS chromatogram with four major peaks that were considerably different than the original analysis. The nizatidine degradation product found in the analysis without diethylamine but at the same retention time as the “polymer” peak had a mass of 187 amu. A reaction with the acid moiety of this product and diethylamine may account for the observed 171 mass unit increments observed in some of the diethylamine containing spectra (Figure 5).
Figure 1. Structure of Nizoldine

Figure 2.

Figure 3.

Figure 4.

Figure 5.

ION AT M+H=187
M+H=345

N+H = 416
APPLICATIONS OF THERMOSPRAY LC/MS AND TANDEM MASS SPECTROMETRY
IN THE CHARACTERIZATION OF NUCLEOSIDES

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More that 80 different nucleosides are presently known in RNA and DNA. They can undergo chemical changes and adduct formation on exposure to certain toxins and carcinogens (1). A number of nucleoside analogs also possess activity against replication of viruses and are thus chemotherapeutic agents (2). Hence it is desirable to have a method available for analysis of nucleosides with high sensitivity and specificity. In this study we report the thermospray mass spectral data on a number of nucleosides. Since thermospray ionization does not provide extensive fragmentation, tandem mass spectrometry was used in combination with thermospray to obtain structural information.

MATERIALS AND METHODS

Materials

The nucleosides were purchased from Sigma Chemical Co. (St. Louis, MO). Fluorothymidine (FLT) was provided by American Cyanamid (Pearl River, NJ). All other chemicals were reagent grade and solvents were HPLC grade.

Thermospray Mass Spectrometry

Mass spectral data were obtained on a VG Trio-3 triple quadrupole mass spectrometer. The HPLC effluent was introduced in the ion source of the mass spectrometer through a heated capillary. The HPLC system consisted of a Hitachi L-6200 pump, and a Valco C6W injector. The mobile phase was Methanol:0.01 M ammonium acetate (20:80; v/v) at a flow rate of 1 ml/min. Direct loop injections were used for obtaining the spectra. Thermospray conditions were optimized for adenosine by proper selection of capillary temperature, source temperature and voltage of the repeller electrode.

Tandem Mass Spectrometry

Tandem mass spectrometric data were obtained by selection of the protonated molecular ion of the nucleoside through the first quadrupole (Q1), and allowing it to undergo collision aided decomposition with Argon in the collision cell, which is an RF only quadrupole (Q2). The ions from the collision cell were mass analyzed by quadrupole (Q3) and were detected on a photomultiplier detector at 400 volts. The collision gas pressure and collision energy were optimized to obtain maximum fragmentation. The collision cell gas pressure was 5.0 E-6 torr and collision energy was 30 eV.

RESULTS AND DISCUSSION

Thermospray mass spectra of nucleosides gave intense pseudo-molecular (MH⁺) ions together with structurally informative fragment ions. The pseudomolecular ions were subjected to collision aided dissociation (CAD), and the mass spectra of the products gave daughter ions corresponding to the nucleic acid base (BH₂⁺) and sugar (S⁺). The relative intensities of the major ions observed in the CAD/MS spectra of the pseudo-molecular ions of the nucleosides are shown in figure 1.

(a) Ribonucleosides

Thermospray mass spectra were obtained on 3 ribonucleosides adenosine, cytosine and uridine. The thermospray mass spectrum of ribonucleosides showed that the protonated molecular ion MH⁺ was the base peak in the spectrum and that there was very little fragmentation corresponding to the BH₂⁺ ions for the nucleic acid base in the nucleoside. The MS/MS spectrum of the protonated molecular ions showed the BH₂⁺ ion as the base peak, and an ion at m/z 133 corresponding to the sugar (S⁺) in addition to the protonated molecular ion.
(b) Deoxyribonucleosides

Thermospray mass spectra were obtained on three deoxyribonucleosides 2'-deoxy adenosine (2DA), 2'-deoxycytidine (2DC) and thymidine (TYM). Protonated molecular ions were base peaks in the spectrum except for thymidine in which the BH$_2^+$ ion at m/z 127 was the base peak. The S' ions were observed at m/z 117 except in 2DC. The CAD mass spectrum of Thymidine had a base peak at m/z 127 (BH$_2^+$) and also ions at m/z 117 (S') and 243 (MH$^+$).

(c) Dideoxyribonucleosides

Thermospray mass spectral data were obtained on four dideoxyribonucleosides dideoxyadenosine (DDA), dideoxycytidine (DDC), 3'-deoxythymidine (3DT) and dideoxyuridine (DDU). The thermospray mass spectra of DDU and 3DT showed excessive fragmentation and the base peak in their spectra was S' ion at m/z 101. The thermospray mass spectrum of DDC showed the protonated molecular ion at m/z 212 as the base peak together with an ion at m/z 112 (BH$_2^+$). The CAD mass spectra of the protonated molecular ion of DDC at m/z 212 showed the BH$_2^+$ ion at m/z 112 as the base peak and also ions at m/z 101 (S') and 212 (MH$^+$).

(d) 3'-substituted nucleosides

Thermospray mass spectra were obtained on two 3'-substituted anti-AIDS nucleosides 3'-azidothymidine (AZT) and 3'-fluorothymidine (FLT). Both of these nucleosides showed MH$^+$ ions as the base peak in their spectrum. The thermospray mass spectrum of AZT showed ions at m/z 127 (BH$_2^+$), 285 (MNH$^+$) in addition to the protonated molecular ion at m/z 268, which was also the base peak in the spectrum. The CAD mass spectra of protonated molecular ion of AZT at m/z 268 showed BH$_2^+$ ion at m/z 127 as the base peak together with ions at m/z 142 (S') and 268 (MH$^+$). Thermospray mass spectrum of Fluorothymidine (Figure 2a) showed MH$^+$ ion as the base peak at m/z 245, together with ion at m/z 262 corresponding to the MNH$^+$ ion. The CAD mass spectrum of MH$^+$ ion of FLT at m/z 245 (Figure 2b) showed BH$_2^+$ ion at m/z 127 as the base peak together with ions at m/z 119 (S') and 245 (MH$^+$). In summary, the CAD mass spectra of nucleosides show S' peaks, characteristic of the sugar part of the nucleosides and BH$_2^+$ peaks characteristics of the nucleic acid base.

REFERENCES


Figure 1

Figure 2
THE DIRECT ANALYSIS OF DRUG CONJUGATES USING LC-MS

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Introduction
In our experience, conjugated drug metabolites often undergo extensive breakdown in the thermospray (TSP) interface, presumed to be due to the thermal instability of such compounds. In an attempt to overcome this problem we have modified our methodoloy to allow the use of lower temperatures on the TSP interface and thus improve the yield of pseudo-molecular ions of the intact conjugated drug or metabolite. Additional data, not presented here, has also demonstrated increased sensitivity by adopting this strategy. Two Glaxo compounds, GR50360 and GR55721, are used to exemplify the benefits accruing from our approach for the analysis of conjugates.

GR50360

GR55721

Methods
The metabolites of GR50360 were purified from samples of rat, dog and human urine. Deuterated-GR50360 was used to assist in these studies. The metabolite of GR55721 was prepared by incubation with rat liver S9 fraction and purified by solid-phase extraction.

Reversed-phase chromatography was performed on a C8 column using mobile phases consisting of methanol and water each containing ammonium acetate (0.02M) at a flow rate of 0.5ml/min. Methanol containing ammonium acetate (0.02M) was added post-column at the same flow rate.

Mass spectrometric analysis was carried out using a Finnigan 4500 instrument fitted with a Finnigan thermospray interface, including the discharge ionisation facility.

Results
1. Metabolites of GR50360
Analysis of two prominent metabolites using full scan, positive ion monitoring gave the following spectra:-

METABOLITE A

METABOLITE B
The ions at m/z433 and 293 are the respective ammoniated cations of the two conjugates. The ion at m/z196, base peak for metabolite A, is equivalent to the protonated cation of GR50360 and arises as a result of the thermal breakdown of the conjugates during the thermospray process. Additional data from negative ion monitoring allowed the molecular weights to be assigned as 415 and 275 and the following structures to be proposed:

The structure for the carbamoyl glucuronide was supported by the discharge ionisation spectrum which showed fragments at m/z222 and 240, characteristic of the presence of a carbamate function on GR50360.

2. Metabolite of GR55721

TSP analysis of the major in-vitro metabolite of GR55721 gave the following spectrum:

The spectrum shows a strong protonated molecular cation at m/z519 and a weak thermal fragment ion at m/z246. This data is characteristic of a glutathione conjugate of GR55721, molecular weight 518.

Conclusions

1. Post-column addition of methanol permits the use of lower temperatures on the TSP interface.
2. The use of lower TSP temperatures reduces the extent to which thermally sensitive compounds undergo thermal degradation.
3. Routine use of the modified approach has resulted in greater success in determining the identities of conjugated drug metabolites.
4. It has also been observed, during these and other studies, that enhanced TSP sensitivity is obtained using high proportions of methanol, typically greater than 60%, in the mobile phase.
5. The use of post-column addition of methanol permits the consistent use of methanol in proportions greater than 50%. This in turn significantly reduces the effect of gradient elution on the TSP performance.
The practical use of Thermospray (TSP) HPLC-MS for the analysis of prostaglandins (PGs) and other eicosanoids in biological matrices is dampened by its low structural information and high detection limits (DLs). Derivatization has been used in order to lower DLs as well as to obtain functional information (1-4). Methoximation of oxo groups afford no significant improvement in DLs due to the extensive solvolysis and adsorption of the derivatives in the hot interface. Despite this, these derivatives could be of interest to obtain functional and structural information (5). Methoxime derivatives of prostaglandins with keto groups show spectra with important signals derived from the loss of the methoxiamine group (2). In contrast, methoximes from aldehyde groups show preferently losses of CH$_3$OH (3) and this behavior allows these two oxo groups to be differentiated. Methoximes could also be of interest in the analysis of compounds showing liquid-phase equilibria implying an oxo group (4).

In the analysis of prostaglandin F$_2$-$\alpha$ (PGF), 6-oxo-PGF$_2$-$\alpha$ (6KF), 2,3-dinor-6KF (DKF) and some of their chemical derivatives (Fig.1), some analytically important fragmentation derived signals were observed. In addition, these three prostanoids, bearing in common a 1,3 dihydroxy moiety in the cyclopentane structure, show important chromatographic and spectrometric differences.

**EXPERIMENTAL**

- Methylation and methoximation were carried out as described (3). No purification was performed prior to chromatography.
- A 0.1 M ammonium acetate (AMAC) buffer (pH 4, formic acid) was used for TSP. The mobile phase consisted of AMAC/MeOH or AMAC/ACN mixtures. LC flow-rate was 1 ml/min.
- Chromatography was carried out on 5 micron (15x0.4 cm) or 3 micron (6x0.46 cm) Spherisorb ODS-2 reverse phase columns.
- Mass spectrometer.- A Hewlett Packard (HP) 5988A quadrupole instrument provided with a TSP source and interface (also from HP) was used. Spectra were recorded at 3 different source temperatures (200, 250 and 300 °C). In the filament on mode, electron energy was set at 250 V. The TSP interface temperature was normally adjusted at 110 °C.

**RESULTS AND DISCUSSION**

**MASS SPECTRA.**- The TSP spectra of these compounds are dominated by signals that can be described by:

\[
[M-H+xBA+(H$_2$NOCH$_3$)$_n$]-n(H$_2$O)]^+ \quad \text{(positive ion mode)} \\
[M-H+xBA-(H$_2$NOCH$_3$)$_n$]-n(H$_2$O)]^- \quad \text{(negative ion mode)}
\]

where M is the prostagland molecule and B and A a base and an acid from the eluent buffer, respectively. The integer n ranges from 0 to 3 or 4 and x,y (y is only applied for methoximated derivatives) from 0 to 1. Ion assignments can be made taking in account the MW shift between the compounds assayed and the spectra obtained in different TSP buffers. The relative abundances of these ions are dependent on the particular structure as well as on source and interface temperatures and eluent composition.

The differences observed in the spectra of these compounds (Table I) point to the formation of different ion structures. It is known that 6KF and DKF show various equilibrium forms in solution derived from the nucleophilic attack of
the C9 hydroxy group to the ketone (Fig 1). The presence of these structures in the gas phase as the more stable ionic species is in agreement with the different base peaks observed for the prostanoids assayed (Table I). The [M+H-HjX]+ base peak of some of these compounds can be explained by the formation of a stabilized cation from II.

**TABLE I. Chemical structures of the compounds and base peaks observed in their TSP mass spectra.**

<table>
<thead>
<tr>
<th>Prostagl.</th>
<th>X</th>
<th>R1</th>
<th>Base Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proetagl</td>
<td>H</td>
<td>CH(CH3)jCOOH</td>
<td>[M+NH4]+</td>
</tr>
<tr>
<td>6KF</td>
<td>=O</td>
<td>CH3(CH3)jCOOH</td>
<td>[M+H-H2O]+</td>
</tr>
<tr>
<td>Methyl esters</td>
<td>H</td>
<td>CH3(CH3)jCOOH</td>
<td>[M+NH4]+</td>
</tr>
<tr>
<td>POFME</td>
<td>=O</td>
<td>CH3(CH3)jCOOH</td>
<td>[M+H-H2O]+</td>
</tr>
<tr>
<td>DKFME</td>
<td>=O</td>
<td>CH3(CH3)jCOOH</td>
<td>[M+H-H2O]+</td>
</tr>
<tr>
<td>Methoximes</td>
<td>NOCH2</td>
<td>CH3(CH3)jCOOH</td>
<td>[M+H-HjNOCH2]+</td>
</tr>
<tr>
<td>6KFMO</td>
<td>=O</td>
<td>CH3(CH3)jCOOH</td>
<td>[M+H-HjNOCH2]+</td>
</tr>
<tr>
<td>Mixed Deriv.</td>
<td>NOCH2</td>
<td>CH3(CH3)jCOOH</td>
<td>[M+H-HjNOCH2]+</td>
</tr>
</tbody>
</table>

(a) ME, methyl ester; MO, methyl oxime.
R2 is always CH3(CH3)jCH3.
(b) Ion source temperature 200-250 °C.

**FRAGMENTATION IONS.** These prostanoids show ions implying a loss of 44 amu which cannot be explained as derived from water losses and/or adduct formation with solvent components. The TSP spectra of C9 and C11 deuterated PGFs, obtained from reduction of PGD2 and PGE2, indicate the fragmentation derived ions are mainly due to the loss of the C10-C11 moiety of the structure.

In addition, the TSP mass spectrum of methoximated 6KF shows a group of ions that could be formed from a fragment of MW 267 amu. These ions are not observed in the spectra of PFG or non derivatized 6KF and can be explained for the fragmentation a to the methoxime group with elimination of a methanol molecule. The extension of these fragmentation processes depends on the composition of the eluent. Although in methanol based eluents abundant fragmentation derived ions are formed, in those based on acetonitrile these signals are of low abundance.

**CHROMATOGRAPHY.** The liquid phase equilibria of DKF (I-III in Fig.2) direct its particular chromatographic behavior and complicate its analysis by TSP. The substitution of the oxo group for the oxime makes more difficult the formation of cyclic equilibrium forms and results in narrower HPLC peaks. Some peak broadening can be observed that can not be correlated with different equilibrium forms or methoxime hydrolysis during chromatography and can be seen in the HPLC-UV profiles. This effect is mainly due to adsorption processes implying hydrolysis or ammoniolysis of the MO group in the hot interface or on the surface of the ion source. Ions like [M+H]+ or [M+NH4]+ show narrow chromatographic signals; unlike this, ions derived from losses of methoximamine, as [M+NH4-CH3ONH2]+, show broad signals. As these ions are carrying the major part of the total ion current due to the derivative, DLs focussing in [M+H]+ and [M+NH4]+ ions are higher than expected for this derivative (DLs of 20 ng for DKFMO and 1 ng for DKFMEMO vs 200 pg for POFME).

**REFERENCES.**

**ACKNOWLEDGMENTS.** This work has been edited using the facilities of the MS Lab at NCTR (Jefferson, AR). We would like to thank Dr W.Korfmacher and the MS Lab staff for their help.
THE CHARACTERIZATION OF A MIXTURE OF SURFACTANTS USING THERMOSPRAY LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY

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INTRODUCTION
Surfactant materials are often used as additives to alter the properties and/or the performance of final products. Analytical characterization of these additives is very difficult because of the varying degree of polymerization and concentrations present. The characterization process becomes even more complicated when the additives are blends of multiple surfactant components.

EXPERIMENTAL
The experimental system (Figure 1) was operated in one of two modes, a column mode (switches S1 and S2 in position A) or a loop-injection mode (switches S1 and S2 in position B). A Rheodyne 7125 injector (I) was used in the loop-injection mode. All LC/MS data were collected under the following conditions unless otherwise noted: 1) Waters 600 MS System Pump (W1) operated at 0.5 ml/min to deliver 55:45 0.002M NH₄OAc in H₂O (pH adjusted to 3.0 with TFAA):ACN; 2) Waters Column Oven (W2) was operated at 35°C using a Spherisorb NH₂ column (100 x 3 mm with 3 μm particles) from Keystone Scientific; 3) Waters UV Detector (W3) was operated at 275 nm with output to the TSQ-700 MS instrument; 4) Waters 590 MS Post-Column Pump (W4) was operated at 0.4 ml/min to deliver 0.005 M NH₄OAc in 80/20 MeOH:H₂O; and 5) Finnigan TSQ-700 MS (TSQ) was operated at a source temperature of 180°C, at a TSP vaporizer temperature of 90°C, and in both the +/− TSP modes. In all cases, 25 μg of sample was injected on column.

SURFACANT COMPONENTS
The sample contains cationic (ethoxylated tallowamine; Aₙ, Bₙ, and Cₙ; where n = 2x; Table I), anionic (ethoxylated alkyl-phenol sulfates; Dₙ, Eₙ, and Fₙ), and nonionic (ethoxylated alkyl-phenols; Gₙ and Hₙ; and polyethylene glycol; Iₙ) surfactant components. All components were detected as indicated (Table I); note that the nonionic components were detected as NH₄⁺ adducts.

LC/UV RESULTS
Initially a weak anion exchange liquid chromatography system using a sodium phosphate buffer system was employed to achieve separation of the surfactant components based upon each component's acidity (Figure 2). Elution times were predicted to increase in the order: cationic, nonionic, and anionic. The data illustrate the separation of four major chromatographic peaks (I-IV). With minor adjustments, the buffer system was converted to an ammonium acetate buffer system for MS compatibility reasons (Figure 3).

LC/UV/TSP MS RESULTS
Reconstructed ion chromatograms illustrate the separation of each component (Figure 3). The first chromatographic peak (I) corresponds to the cationic component which contains three major series of tallowamine ions (Aₙ, Bₙ, and Cₙ; Table I; Figure 4 illustrates a typical mass spectrum). The second chromatographic peak (II) corresponds to the nonionic component which actually contains two series of ethoxylated alkyl-phenol ions (Gₙ and Hₙ; Table I) and polyethylene glycol ions (Iₙ; Table I). The third chromatographic peak (III) corresponds to one anionic component which contains a series of ethoxylated alkyl-phenol sulfate ions (Dₙ; Table I). The fourth chromatographic peak (IV) corresponds to a second anionic component which contains two series of ethoxylated alkyl-phenol sulfate ions (Eₙ and Fₙ; Table I). The series of ions corresponding to Dₙ and Eₙ are empirically equivalent, however the proposed structures are consistent with the elution order of the components based on expected acidities. MS/MS experiments have not yielded any useful data to distinguish these two series of ions.

LC/UV/TSP MS OPTIMIZATION
For all series of ions, increasing the discharge voltage caused an increase in the background level, and an overall decrease in the signal/noise ratio for the ions in each series. The TSP vaporizer temperature optimized at 90°C for all ions in each series. The source temperature (Figure 5) and post column buffer concentration (Figure 6) optimization varied for each surfactant component.
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Figure 1. Experimental System.

Figure 2. Sodium Phosphate Buffer.

Figure 3. Reconstructed Ion Chromatograms.

Figure 4. +TSP Mass Spectrum of Cationic Components (A_n, B_n, and C_n).

Figure 5. TSP Source Temperature Optimization.

Figure 6. TSP Post-Column Buffer Concentration Optimization.

Table I. Surfactant Components

\[
\begin{align*}
A_n & = \text{C}_n\text{H}_{2n+1}\text{N} - \text{(CH}_2\text{CH}_2\text{O})_n\text{H} - \text{H}^+ \\
B_n & = \text{C}_n\text{H}_{2n+1}\text{N} - \text{(CH}_2\text{CH}_2\text{O})_n\text{H} - \text{H}^+ \\
C_n & = \text{C}_n\text{H}_{2n+1}\text{N} - \text{(CH}_2\text{CH}_2\text{O})_n\text{H} - \text{H}^+ \\
D_n & = \text{C}_n\text{H}_{2n+1}\text{N} - \text{(CH}_2\text{CH}_2\text{O})_n\text{H} - \text{H}^+ \\
E_n & = \text{C}_n\text{H}_{2n+1}\text{N} - \text{(CH}_2\text{CH}_2\text{O})_n\text{OH} \\
F_n & = \text{C}_n\text{H}_{2n+1}\text{N} - \text{(CH}_2\text{CH}_2\text{O})_n\text{OSO}_3\text{H} \\
G_n & = \text{C}_n\text{H}_{2n+1}\text{N} - \text{(CH}_2\text{CH}_2\text{O})_n\text{OH} + \text{NH}_3 \\
H_n & = \text{C}_n\text{H}_{2n+1}\text{N} - \text{(CH}_2\text{CH}_2\text{O})_n\text{OH} + \text{NH}_3 \\
I_n & = \text{H}^+ - \text{(CH}_2\text{CH}_2\text{O})_n\text{OH} + \text{NH}_3
\end{align*}
\]
A COMPARISON OF LC/MS TECHNIQUES FOR PHARMACEUTICAL ANALYSIS

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INTRODUCTION

The development of a variety of interfaces for LC/MS offers the analyst a choice of methodologies for characterizing multicomponent samples. A comparison of heated nebulizer atmospheric pressure ionization and ion spray atmospheric pressure ionization using a Sciex API III mass spectrometer, and continuous flow FAB (CF-FAB) using a VG ZAB-HF mass spectrometer for the analysis of pharmaceutical samples is presented. The sensitivity and selectivity of each of these techniques is evaluated for several classes of drugs.

EXPERIMENTAL

Continuous flow FAB spectra were obtained using a VG universal FAB ion source and probe. Flow rates of 5 μl/min into the source were used. Mobile phases contained water and CH3CN, with 5% glycerol. Heated nebulizer atmospheric pressure ionization and ion spray spectra were obtained on a Sciex API III mass spectrometer. Zero grade air was used as the nebulization gas for heated nebulizer atmospheric pressure chemical ionization. Ultra high purity nitrogen was used as the nebulization gas for ion spray. Collisions were 40 eV with argon.

RESULTS

Benzazepine Calcium Entry Blockers are amenable to all three techniques with limits of detection in the low picogram range. The CF-FAB interface provides the lowest limit of detection--50 pg for full scans, and less than 10 pg for SIM experiments. Full scan spectra can be obtained on 100 pg using either the heated nebulizer or ion spray interface. Ion spray SIM and selected reaction monitoring (SRM) experiments have a limit of detection of 50 pg. Ion spray full scan daughter ion spectra require 5 ng of compound.

The CF-FAB mass spectra of mycosamine containing Polyene Antifungals, such as amphotericin, exhibit intense pseudomolecular ions. The full scan limit of detection using this interface is 500 pg. Ion spray mass spectra are similar, but the limit of detection is 2.5 ng. No pseudomolecular ions are observed when the heated nebulizer interface is used.

The ion spray interface provides intense pseudomolecular ions for the analysis of thermally labile Monobactam Antibiotics, such as aztreonam. In the negative ion mode, the full scan limit of detection is 500 pg. Using CF-FAB in the positive ion mode, 5 ng are required for full scan spectra. As would be expected for thermally labile compounds, only thermal decomposition products are present in positive or negative ion spectra obtained with the heated nebulizer interface.

Decalin ring containing Cholesterol Lowering Agents, such as pravastatin and lovastatin, exist as either carboxylic acids or pro-drug lactones. For analysis of the lactone forms, both CF-FAB and heated nebulizer spectra exhibit intense pseudomolecular ions with limits of detection of 500 pg. Structurally informative full scan daughter ion spectra are obtained with 4 ng. The ion spray spectra of these neutral molecules show only (M+NH4)+ at levels of 50 ng.

For the carboxylic acid forms, the lowest limits of detections (500 pg) are obtained by ion spray or CF-FAB. Less than 250 pg can be detected using SIM. Ion spray full scan daughter ion spectra require 10 ng of compound. Limits of detection using the heated nebulizer interface are 100 ng for full scan and 5 ng for SIM.
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ION SPRAY
PRAVASTATIN

(M+H)^+

HEATED NEBULIZER
LOVASTATIN

(M+H)^+

ION SPRAY
NEGATIVE IONS
AZTREONAM

(M-H)^-

CF-FAB
AZTREONAM

(M+H)^+

CF-FAB
AMPHOTERICIN A

(M+H)^+

HEATED NEBULIZER
BENZAZEPINE

(M-H)^-

79 90 29 42

LOVASTATIN (M^H)

9 ng diluted to 19 ml/min
30-50 H2O:CH3CN 40:60

303 307

AZTREONAM (M-H)^-

50 ng injected
7 µl/min
47.5:47.5 H2O:CH3CN glycerol

285 303

ION SPRAY

30 30 H2O.CH3CN

30

367

425

100 75 50 25

422 430 438 446

428 440 448


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An Improved Atmospheric Pressure Ionization Interface for LC/MS on a Bench-Top Mass Spectrometer.

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Although our goal of performing routine LC/MS on a small bench-top mass spectrometer has been demonstrated [1,2], the sensitivity of our first system was compromised by the limited pumping speed. Sensitivity of the atmospheric pressure ionization interface can be increased by increasing the diameter of the sampling orifice and/or skimmer. Although this allows more ions to enter the system, it also places a heavier gas burden on the pumping system. Thus for a simple interface, a very high pumping speed is need. The pumping capacity can be reduced (along with the cost) if one or more stages are added to the interface that permit separation of ions from the neutral gas.

Rather than increasing the pumping speed of the high vacuum analyzer region of our system, we have chosen to increase the ratio of ions to neutral gas molecules in an intermediate rough pumped region. An RF-only quadrupole lens system was selected as the method for transmitting the ions to the mass analyzer while reducing the amount neutral gas entering the analyzer. A diagram of this instrument is shown in Fig 1. The mass spectrometer is a Hewlett-Packard 5790 MSD that has an enlarged analyzer housing and a 330 L/sec turbomolecular pump. All of the ion optics were removed with the exception of the mass analyzer quadrupole and the detector. The ions that enter the analyzer are confined by an RF-only quadrupole lens system that is capacitively coupled to the mass analyzer quadrupole by butting the two sets of rods together separated by 0.001 in mylar spacers.

The ions entering the analyzer in the previous version of this system [3] exited the skimmer of the heated capillary/skimmer [4] interface. In our latest system an intermediate RF-only quadrupole stage has been added. This lens is directly connected to the other RF-only quadrupole via copper/nylon feed-thrus. (Capacitive coupling was also tried but little difference in performance was noticed between the two methods.) This region is pumped with a inexpensive rough pump. Pressures are indicated on Fig 1 for the various regions.

Although the new system demonstrates a sensitivity increase of a factor of 3-5, problems were encountered when the instrument was scanned over more than several amu or when SIM was attempted. The problem was traced to what we believe may be a charging phenomenon. The quadrupole was referenced to ground through a resistor network. When a slight positive voltage was applied to the quadrupole, the system then worked normally. The voltage required seems to have some dependence on the scan rate. This is being studied further.

In order to increase the rate of removal of gas from the intermediate region, a quadrupole was constructed from mesh instead of solid rods. A decrease in sensitivity of a factor of 4 was observed with the mesh rods. Obviously more studies need to be done on both the effects of pressure in this intermediate region as well as the actual quadrupole construction.

LC/MS runs with the new system on a mixture of pesticides at the 50 ng per component level at 3.4 sec scan over the mass range of 10 to 450 demonstrate the utility of the instrument. SIM experiments with LSD at the 250 pg level from a 1 x 100 mm LC column show a factor of 5 fold increase in sensitivity over the previous system. An LOD of 100 pg is now possible. The additional stage can also provide CID type spectra by increasing the potential on the skimmer, although not with the same sensitivity as that which occurs between the capillary and skimmer.

20 cfm Rough Pump 10 cfm Rough Pump
330 Liter/sec Turbo Pump

130-250Vdc

500μm id 0.25 od

Heater

Insulator

1.4 Torr

0.02 Torr

RF-Only Quad

1-2 Vdc

Analyzer Housing

5 x 10⁻⁵ Torr

RF-DC Quad

Moved to show detail

Mylar insulator

Nylon stud

Figure 1
HPLC/MS FOR THE DETERMINATION OF AMINOGLYCOSIDES USING ATMOSPHERIC PRESSURE IONIZATION

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As part of a regulatory effort for determining aminoglycoside residues in tissues and milk of livestock, the U.S. Food and Drug Administration (FDA) requires confirmatory methods based on mass spectrometry. Because of their polar, basic nature and thermal lability in some cases, these compounds cannot be determined directly by GC/MS. However, methods based on HPLC/MS and HPLC/MS/MS could provide direct, on-line identification of these drugs in complex biological matrices. Existing HPLC methods for their determination in animal tissues employ fluorescence detection of derivatized analytes (1). There are few reports of aminoglycoside determinations by HPLC/MS. Considering the nature of these analytes, the unique features of the ion spray HPLC/MS interface make it worth investigating for this application.

Most reported HPLC methods are based on some form of ion chromatography, ion pairing or ion exchange for example, because the aminoglycosides are difficult to retain in the reversed-phase mode, even with an entirely aqueous eluent (2). The majority of reported conditions are not well-suited for use with the ion spray interface due to the presence of high concentrations of non-volatile buffers and alkali salts. Samain et al. have reported the use of volatile acids such as pentafluoropropionic acid (PFPA) as ion pairing agents to separate aminoglycosides in the reversed-phase mode (2). Here we explore this approach for determining aminoglycosides by ion spray LC/MS. The HPLC method is also compatible with a commercial pulsed amperometric detector. Data are presented for six priority compounds: spectinomycin, hygromycin B, streptomycin, dihydrostreptomycin, gentamicin C, and neomycin B in selected ion monitoring (SIM) and selected reaction monitoring (SRM) modes of operation.

EXPERIMENTAL

Separations were developed using a Gradient Pump Module and PAD 4500i pulsed amperometric detector ( Dionex Corp.) on a Spherisorb ODS-2, 4.6 mm i.d. x 100 mm column at a flow rate of 1 mL/min. HPLC/MS work was done with a Spherisorb ODS-2, 2 mm i.d. x 100 mm column at a flow rate of 200 µL/min using a Waters Model 510 pump. Mobile phases consisted of 8% acetonitrile (ACN) in 20 mM PFPA for isocratic separation of four of the analytes and 22% ACN in 20 mM PFPA for isocratic separation of gentamicin and neomycin. Column effluent was split with a PEEK tee such that 20-40 µL/min reached the ion spray interface that was maintained at 3.4 kV. The mass spectrometer, a Sciex TAGA 6000E upgraded to an API III, was used in the positive ion mode. Collision-induced dissociation (CID) experiments were accomplished with argon at 180 x 10^12 atom/cm² and collision energies of 76 eV (laboratory frame). Control bovine kidney was fortified at the 20 ppm level with four of the analytes and extracted using the matrix solid phase dispersion (MSPD) technique (3) with modifications for this class of compounds (4). Analytes were eluted from the MSPD column with 1 mL water and 8 mL 0.1 N sulfuric acid. An aliquot of the 9-mL eluate was concentrated by a factor of 4, neutralized with ammonium hydroxide, and 10 µL injected onto the HPLC column.

RESULTS AND DISCUSSION

Although increasing concentrations of PFPA in the mobile phase were found to decrease ion spray response, a concentration of at least 10 mM is necessary for adequate chromatographic resolution and 20 mM provided the best separation efficiency on the Spherisorb ODS-2 column. The gentamicin C components and neomycin B are more highly retained than the other four analytes and cannot be eluted unless a gradient program is used. Here, we have used two separate isocratic methods. Improvements are needed with respect to resolution of the gentamicin C components and peak tailing of the gentamicins and neomycin B.

Under these conditions doubly charged ions predominated for all analytes except spectinomycin for which singly charged ions were observed. The mass spectra exhibit high chemical noise especially at the relatively low m/z of the doubly charged ions. Extracted ion current profiles from ion spray LC/MS in SIM mode for four analytes are shown in Figure 1. Confirmation is accomplished by LC/MS/MS in the SRM mode. CID spectra of all
compounds except spectinomycin and hygromycin B contain at least three abundant product ions which can be monitored for confirmation. The detection limit for dihydrostreptomycin by LC/MS/MS SRM was found to be approximately 5 ng injected on-column. Results for gentamicin and neomycin with LC/MS/MS SRM are shown in Figure 2. A bovine kidney extract fortified at 20 ppm of spectinomycin, hygromycin B, streptomycin, and dihydrostreptomycin was analyzed by LC/MS/MS in the SRM mode and no matrix interferences were observed at this level. See Figure 3. This concentration corresponds to injection of 40 ng of analyte on-column assuming total extraction recovery. Given detection limits of approximately 5 ng for this method, it should be possible to reach the desired low to sub-ppm levels in bovine kidney by further concentration of the 9-mL MSPD eluate and by increasing extraction recovery.

ACKNOWLEDGEMENTS

The authors thank the U.S. FDA for funding this work under agreement # FD-U-000577-02 and Dionex Corp. for the generous loan of the pulsed amperometry system. Mr. Mark Mercer and Dr. Phillip Kljak are acknowledged for many helpful discussions.

REFERENCES

Antibiotics are commonly used for the prevention and treatment of disease in farmed animals raised in crowded conditions. A major concern is that residues of these compounds may be present in the final food product if proper withdrawal times for treated animals have not been strictly enforced. Tolerance levels (typically 100 ppb (ng/g)) must be enforced through monitoring programs.

Immunoassay techniques are ideal for the routine screening of food products for antibiotics. However, instrumental methods of analysis are usually required for confirmation, especially in a regulatory situation, since immunoassays are often subject to false positives. Legal cases usually demand a spectroscopic proof of identity, with mass spectrometry being the most accepted. Spectroscopic techniques are also essential for the identification of metabolites of drugs which may also be of concern in monitoring programs. Most antibiotics and their metabolites are difficult to analyze by GC-MS, since they are polar and thermally-labile. The lower molecular weight compounds can be converted to volatile derivatives, but such analyses are complicated and require extensive sample clean-up. All antibiotics can be analyzed by HPLC, however. Therefore, the combination of liquid chromatography and mass spectrometry (LC-MS) is clearly the most desirable method for confirmatory determinations.

We have evaluated various ionization and interfacing methods for the LC-MS analysis of antibiotics used in the aquaculture industry, and tested their application to the confirmation of residue levels in farmed salmon. The methods that have been compared include: ion-spray (ISP), thermospray (TSP), particle beam interface (PBI, both EI and CI), and moving belt interface (MBI, both EI and CI). The antibiotic and antimicrobial drugs that have been examined include: oxytetracycline (OTC), erythromycin (ETM), penicillin G (PCG), oxolinic acid (OLA), sulfamerazine (SMR), sulfamethazine (SMT), Romet-30® (sulfadimethoxine (SDM) and ormetoprim (OMP)), and Tribriessen® (sulfadiazine (SDZ) and trimethoprim (TMP)).

A comparison of the spectra generated for one compound, oxytetracycline (OTC), by the different techniques is provided in Figure 1. Ion-spray, an atmospheric pressure ionization technique, provided the greatest sensitivity and ease of interfacing with LC. Detection limits for standards ranged from 5 to 50 pg for selected ion monitoring; good spectra could be acquired on 1 to 10 ng of standard. Thermospray was the next most sensitive with detection limits of 1 to 10 ng with selected ion monitoring; good spectra required 50 to 500 ng. The particle beam and moving belt interfaces were able to provide good chemical ionization spectra for most of the compounds, but required levels ranging from 100 ng to 5 µg. Only the potentiators, OMP and TMP, gave good EI spectra with either of these interfaces. All other compound fragmented extensively or decomposed thermally.

Thermospray, particle beam and moving belt interfaces did not have the sensitivity required for the analysis of actual fish tissue samples with the conventional extraction methods used for HPLC screening. Only ion-spray LC-MS was able to provide detection limits at the ppb level (in salmon flesh) for all compounds tested. Excellent quantitation was also possible with ion-spray LC-MS. Tandem mass spectrometry on the triple quadrupole SCIEX API-III instrument was found to be useful for additional confirmation of structures and for selective detection of analytes with selected reaction monitoring. Figure 2 presents the ion-spray LC-MS analysis of salmon flesh contaminated with 160 ppb (ng/g) of OTC.

* NRCC # 32984
Figure 1. Mass spectra of OTC with different techniques.

Figure 2. Analysis of salmon flesh contaminated with OTC.
Steroids and prostaglandins play important roles as hormones in many biological systems. Water soluble metabolites of these compounds have recently been implicated as prime candidates for fish sexual pheromones [1]. Herring milt appears to contain such a spawning pheromone that would have practical significance in the "roe-on-kelp" industry [2].

Ionspray (ISP) is a recently developed atmospheric pressure ionization (API) technique, well suited for the mass spectral analysis of polar and thermally labile molecules of marine origin. The ISP technique is proving to be a practical interface for the routine coupling of liquid chromatography with mass spectrometry (LC-MS) [3]. We have developed LC-API-MS methods for the analysis of mixtures of underivatized steroids, their glucuronide and sulfate conjugates, and prostaglandins. Under positive ion ISP conditions the steroidal compounds produce abundant protonated molecules which are ideal for the detection of trace levels by LC-MS using selected ion monitoring and also as precursors in tandem mass spectrometric (MS-MS) experiments for structural confirmation. The ISP-MS and ISP-MS-MS spectra of testosterone glucuronide (100 ng) obtained by flow injection analysis are presented in Figure 1a and 1b, respectively. The MH+ ion of the conjugated steroid is clearly observed at m/z 465 in Figure 1a and this ion undergoes collision-induced dissociation to yield a characteristic product ion at m/z 289 (Figure 1b) corresponding to the parent testosterone. The prostaglandins were best analyzed in the negative ion mode, generally providing abundant deprotonated molecules, [M-H]-.

Figure 2a shows the analysis of a mixture of both free and conjugated steroids by LC-MS using selected ion monitoring. The separation was achieved on a 2.1 mm i.d. Vydac 201TP column using a mobile phase of aqueous acetonitrile containing 0.1% trifluoroacetic acid (TFA) at a flow rate of 200 μL/min. A linear gradient of 5% to 60% acetonitrile over 50 minutes was used. The reconstructed ion chromatogram is comprised of the MH+ ions of all the compounds. Similar results were obtained for mixtures of prostaglandins in the negative ion mode using acetic acid instead of TFA.

We have applied LC-MS and LC-MS-MS techniques to the screening of bioactive extracts from herring milt and testes for likely pheromone candidates. Figure 2b shows the ion chromatogram of m/z 465 extracted from the full-scan LC-MS analysis of a herring milt extract. The chromatographic peak at around 32 mins corresponds to the correct retention for the testosterone glucuronide standard (Figure 2a). LC-MS-MS experiments using selected reaction monitoring provided additional supporting evidence. Other steroidal compounds have been identified in this manner and have been subsequently isolated and their structures confirmed by high resolution mass spectrometry using fast atom bombardment and also nuclear magnetic resonance. We are currently assessing the bioactivity of these compounds.

Figure 1

Figure 2

1. Cortisol
2. Cortisone
3. 17,20-dihydropregesterone glucuronide
4. Testosterone glucuronide
5. 11-keto testosterone
6. 4-pregnene-17,20,21-triol-3-one
7. 17,20-dihydropregesterone
8. Testosterone

b) m/z 465
Atmospheric pressure ionization mass spectrometry (API-MS) is rapidly becoming a universal detection system for the separation sciences. The ionspray interface (ISP) introduced by Henion and co-workers in 1982, has had a tremendous impact on the development of combined liquid chromatography mass spectrometry (LC-MS) [1]. Unfortunately, one of the problems associated with "soft" ionization techniques like ISP and the earlier developed thermospray (TSP), is that common MS reference compounds, such as PFK, cannot be used to calibrate the mass spectrometer. Polymers, such as polypropylene glycol (PPG), are generally used as calibrants in both TSP and ISP. Unfortunately, PPG mixtures are not the most suitable candidates for reference compounds, causing rapid contamination of the ion source in TSP [2,3]. In addition, it is often difficult to identify individual PPG oligomer peaks in the complex spectra obtained with the mixtures used to cover the entire mass range. There is a clear need for a more universal calibration method in ISP.

Cesium iodide cluster ions (Cs_{n}ln-i^{+}), and other alkali metal halide salts, are routinely used as mass calibration standards in fast atom bombardment (FAB) and liquid secondary ion mass spectrometry (LSIMS). However they have not thus far been amenable to any of the spray techniques. We report here on the formation of these and related cluster ions by ionspray ionization, under conditions typical of those used in LC-MS analyses of peptides and proteins. The spectrum shown in Figure 1 was obtained by flow injection analysis of 5 µL of a 0.1 mg/mL solution of CsI in water into a 10 µL/min flow of aqueous acetonitrile (50:50) containing 0.1% trifluoroacetic acid. Similar spectra were obtained, though at lower intensities, at flow rates of up to 50 µL/min. The well-known Cs_{n}ln-1^{+} ion series is observed (the Cs^{+} peak at m/z 133 was extremely intense, and has been omitted from the Figure). In addition, unexpected satellite peaks at lower mass are also observed for each of the Cs_{n}ln-1^{+} clusters. As shown more clearly in the insert in Figure 1, the spacings of these satellite peaks are all 14 Da, and the number of satellite peaks observed increases with the mass of the CsI cluster to a maximum value given by the number (n-1) of iodide ions in the particular cluster ion. These satellite peaks correspond to progressive replacement of iodide (127 Da) by the trifluoroacetate anion (TFAc; 113 Da) present in the mobile phase. Such a substitution for iodide is reminiscent of that observed for FAB ionization of CsI in glycerol-water mixtures, where the glyceride anion C_{3}H_{7}O_{5}^{−} substituted for one iodide per cluster ion [4]. In addition to the satellite peaks described above, other peaks due to cluster ions of the Cs_{n}ln-1^{+} series with mobile phase molecules are observed. For example the peak at m/z 534 in Figure 1 corresponds to the cluster [Cs_{2}I + (MeCN)_{3} + H2O]^{+}. Table 1 lists the molecular formulae and accurate masses of selected calibrant ions observed in this work.

An alternative approach to calibration for LC-MS is to use the mobile phase itself. It has been shown previously that acetic acid-ammonia cluster ions [(CH_{3}COOH)_{x} + (NH_{3})_{y} + NH_{4}]^{+} can be generated under certain conditions in TSP ionization to provide a convenient mass calibration over the range 100-1000 Da [2]. This range has also been extended to higher mass by the use of perfluorinated alkyl acids in place of acetic acid [3]. We have developed similar approaches for ISP using solutions of aqueous acetonitrile or methanol containing small percentages (0.1-5%) of acetic,
formic or trifluoroacetic acids which are commonly used as mobile phases for LC-MS in our laboratory. By optimization of the source parameters, intense mobile phase cluster ions can be selectively generated across the mass range (m/z 100-1500) which are suitable for calibration purposes.


Table 1 Molecular formulae and accurate masses of selected observed calibrant ions

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<th>Formula*</th>
<th>Mass</th>
<th>Formula*</th>
<th>Mass</th>
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*TFAc = CF3COO-

Figure 1 Ionspray spectrum of CsI obtained by flow injection analysis
There is growing interest and awareness in the role played by sugars and other carbohydrates in biological systems. The analytical determination of these saccharides presents a significant challenge, however, since GC-MS cannot be used because of their thermal lability, and since LC is problematic because they have no chromophore. Several mass spectrometric techniques, have been successfully applied to study saccharides, so there is great interest in developing LC-MS methodology for this purpose. Thermospray LC-MS has been used to determine some mono- and di-saccharides, but it has not been successful for tri- and higher saccharides.

We have explored the use of an API (atmospheric-pressure ionization) LC-MS interface for this application, taking advantage of chemical ionization processes possible with this interface. Because of the high frequency of intermolecular collisions that occur in the APCI source, adduct ions and cluster ions are formed more effectively than is the case with ionization mechanisms that operate at low pressures or in a vacuum. Specifically, we used the attachment of chloride ions to hydroxyl groups in saccharides within the APCI source.

The work was carried out on an LC-MS instrument incorporating an atmospheric-pressure chemical ionization (APCI) interface similar to ones described in earlier work (1,2). The LC eluent is sprayed from a heated nebulizer, passes a vaporizer section where it is fully converted into an aerosol, and then it passes a corona discharge formed at the tip of a needle, all at atmospheric pressure. The ions enter an reduced pressure stage first and then into the high vacuum of the quadrupole analyzer. A voltage applied across the two apertures at the entrances to these two regions provides sufficient kinetic energy to strip away the solvent shell around analyte molecules as they pass through the reduced pressure region. Higher values of this “drift voltage” result in fragmentation of analytes (by collision-induced dissociation), and structural information about the analyte can be deduced from the fragmentation pattern.

The ionization process is initiated when a hydroxy hydrogen is stripped away from its oxygen atom in the corona discharge. The resulting alkoxyl or hydroxyl group then extracts a hydrogen from chloroform (added at a low level to the LC mobile phase), which in turn yields Cl⁻ ions as it falls apart. These chloride ions will interact with the hydroxy groups in a saccharide, but the resulting complex has too much internal energy to be stable. This excess energy is lost through the frequent molecular collisions occurring at atmospheric pressure, stabilizing the ion-molecule pair, that can then be detected in the mass spectrometer. Chloroform is a key factor; trials with CCl₄ and CH₂Cl₂ yielded sucrose peaks orders of magnitude smaller. The appearance of vacancy peaks in mass chromatograms, at mass values corresponding to Cl⁻ and to Cl⁻ complexed with one and with two methanol molecules (see Fig. 1) provide supporting evidence for the involvement of these as reagent ions in the APCI source.

Fig. 2 shows the quantitative analysis of several saccharides, where peak area is plotted as a function of the amount injected. In the top panel, no major difference resulted for mobile phase chloroform content ranging from 0.5% to 5%, but the signal intensity decreases when the chloroform content was dropped to 0.05%. Good linearity was demonstrated for between 1 and 100 ng injected (with a 4.0 x 250 mm LiChrosorb NH₂ column) for most saccharides, as shown in the lower panel of Fig.2. Fig. 3 shows the mass spectra obtained with chloride attachment LC-negative ion-APCI-MS for 1 μg each (by flow injection) of underivatized D-arabinose, D-glucose, and D-galactose.

We have injected several monosaccharides and disaccharides with good results. This chloride attachment chemical ionization method is easily implemented, requiring just the addition of a small amount of chloroform to the mobile phase in reversed-phase separations. Detection limits are attractive, below 1 ng (glucose) by SIM mode.
Quantitative Analysis by Chloride Attachment LC/NI-API-MS.

Peak area as a function of amount of substances studied.

- For different amount of chlorination mobile phase additive (for glucose injected)
- For several substances (in 5% CHC13/acetonitrile)

| Column: LChrom NH2 4.6x250 mm; Mobile phase: 5% CHC13, 95% methanol | Flow rate: 3 mL/min; Nebulizer temp: 170°C, Omvoltage: 130 V |

Chloride Attachment LC/NI-API-MS Mass Spectra

for 1 μg each of arabinose, glucose, and galactose.

- Flow injection: Mobile phase: 5% CHC13, 95% methanol; Flow rate: 3 mL/min; Nebulizer temp: 170°C, Omvoltage: 130 V |

Atmospheric pressure chemical ionization (APCI) and Electrospray ionization (ESI) are complementary ionization techniques for liquid chromatography-mass spectrometry. Together these two ionization techniques can allow the analysis of most chemical classes. The range of molecules that can be efficiently ionized by each technique overlaps greatly, with APCI giving best sensitivity for molecules that have relatively low polarity to those with moderately high polarity. ESI yields the best sensitivity for ionic species, however, good ionization efficiency can be obtained for moderately polar molecules. ESI also gives the benefit of multiple charging, thus allowing the determination of high molecular weights on a compact quadrupole mass spectrometer.

In an atmospheric pressure ionization mass spectrometer, the ionization source is outside of the mass spectrometer. This allows very low maintenance on the high vacuum system since most of the solvent is removed at atmospheric pressure. Since the analyte is then expanded into a vacuum, adiabatic cooling occurs, causing analyte-solvent clusters to be formed. A collisionally induced dissociation (CID) technique is used to desolvate the analyte. Both ESI and APCI are soft ionization techniques, forming only molecular ions. By increasing the energy of the CID (Drift Voltage), one can cause easily controllable fragmentation of the analyte ions with APCI or ESI.

Two atmospheric pressure ionization sources - Electrospray ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) have been developed for a Hitachi M-1000 single quadrupole mass spectrometer. These ion sources have been designed to be readily interchanged on the mass spectrometer, switching between the two ionization modes requires no tools and only a few minutes. The LC/MS system is comprised of a Hitachi L-6200 gradient HPLC pump, a Rheodyne injection valve, ionization source (either ESI or APCI), Differentially pumped CID region, single quadrupole mass analyzer, electron multiplier, and an LC/MS data station.

The APCI source uses a heated nebulizer, followed by a vaporizer and a corona discharge needle. Ionization occurs by gas phase ion-molecule reactions between the solvent (which acts as the chemical ionization reagent) and the analyte. Collisionally induced declustering occurs in an intermediate pressure (~1 torr) region. The bare analyte ions are then mass analyzed. This source allows flow rates from 0.2 ml/min to 2 ml/min. There is little sensitivity change due to nebulizer temperature variations, allowing multiple solvents to be used in gradient elution chromatography runs. The CID region consists of multiple apertures, each 0.3 to 0.5 mm id, to which a voltage differential is applied (Drift Voltage). The ESI source consists of a teflon inlet tube to a 0.1 mm id capillary tube to which 3-6 kV is applied. Nitrogen flows concentric to the capillary and both assists in the nebulization of the eluent, but also causes evaporation of the solvent from the charged, nebulized droplets. This design obtains efficient analyte charging and solvent removal without the use of a curtain gas. The analyte is ionized in the liquid-phase and ions are evaporated or ejected from the droplets. The capillary is off axis from the CID inlet to improve signal to noise by minimizing solvent that enters the mass spectrometer. Ions are directed into the CID region by the high voltage differential between the capillary and the inlet aperture. The ESI source uses the same electronics as the APCI source, including the HV supply. The CID region is also shared. The ESI source mounts to the front of the CID region by means of thumb screws.

In the data presented, examples are given for APCI, showing the use of ion selective detection of steroids, and the effect of the drift voltage for controlling the level of fragmentation from none to complete fragmentation. Examples are also given for ESI showing the determination of various proteins, and also an example of the use of ESI for smaller molecules, again demonstrating the use of controlled fragmentation. A calibration curve is presented showing linear response down to 100 pg (680 femtomoles of acetylcholine).
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Fig. 1 APCI mass spectra of Phenylbutazone showing the effect of changing the Drift Voltage on the collision induced dissociation region.

Fig. 2 Mass Chromatograms of a Mixture of Testosterone and Progesterone using APCI. A) 2 ng of Testosterone and 1.1 ng of Progesterone B) 40 ng of Testosterone and 22.5 ng of Progesterone. 90% methanol / 10% water, 1 ml/minute. SIM detection of m/z 289 and 315.

Fig. 3 Electrospray mass spectrum of Cytochrome C from chicken heart (MW=12,222)

Fig. 4 Electrospray mass spectrum of Glyceraldehyde-3-Phosphate Dehydrogenase from porcine muscle (MW=35,700).

Fig. 5 Electrospray mass spectra of Acetylcholine Chloride showing the effect of Drift Voltage on fragmentation.

Fig. 6 Calibration plot for Acetylcholine Chloride. Detection limit is 680 femtomoles (100 pg).
The applications of capillary electrophoresis (CE) are expanding rapidly, and a number of commercial instruments are now available. As applications have developed, the advantages of CE combined with mass spectrometry (MS) have been increasingly recognized. The first CE-MS was based upon an electrospray ionization (ESI) interface. Alternative CE-MS interface designs have also been reported based upon continuous flowing fast atom bombardment (CF-FAB) mass spectrometry. However, the ESI methods appear to offer clear advantages in most cases due to better sensitivity, reduced background, applicability to higher molecular weight compounds, and interface designs which do not require long transfer lines or incur a pressure drop across the capillary. To date, none of the CE-MS instrumentation has effectively utilized the full capabilities of the automated CE instruments now commercially available.

Our aim has been to exploit all the advantages of automated CE instrumentation, and particularly provisions for capillary temperature control by modifying a Beckman P/ACE 2000 capillary electrophoresis instrument to accept an ESI interface; to evaluate the performance of the combined instrument on separations of biological materials, and to demonstrate the capabilities of mass spectrometry combined with CE. The Beckman P/ACE 2000 system, as sold by the manufacturer, was too large to be mounted close to the inlet of our mass spectrometers. We opted to separate the P/ACE 2000 system into two subunits according to their practical and logical functions. The autosampler, interface block, optics module and detector were removed from the mainframe and mounted in a box as a separate "experimental module". The system power supplies (high and low voltage), controller, keyboard, temperature and pressure controls were left on the main frame as a "control module". The interface block of the instrument (which holds the capillary cartridge) was modified by the addition of an extension. The extension is sealed by septa at each end, has separate inlet and outlet lines for the cooling fluid and bolts onto the block immediately below the normal capillary exit. With the downstream septa removed the capillary can be threaded through the extension without removing it from the instrument. With this arrangement, a capillary of approximately 35 cm additional length is required. This length will be reduced in the future by direct incorporation of the electrospray interface at the end of the extension.

The performance of the combined CE-MS system was evaluated in these preliminary studies with a mixture of myoglobin proteins (M~17 kD). The separation was done in 10 mM TRIS buffer at pH 8.3 with an electric field strength of 120 V/cm. Approximately 100 femtoles per component was injected onto the capillary column. The results show that the ESI-MS interface has not degraded the quality of the separation by broadening of the peaks due to the electrospray source. In fact, these peak widths are sufficiently narrow to exceed current capabilities when signal intensities are low and slower scan speeds required. For targeted compound analysis (using single ion monitoring) peaks of 0.1 seconds in width could be detected.

An example of a separation of a more complex mixture is a tryptic digest of tuna cytochrome C. The digest was initially infused into the mass spectrometer and an ESI mass spectrum recorded. The most prominent ions in the spectra were identified and these were monitored in a multiple ion detection CE-MS experiment.

The results of a single ion monitoring experiment are shown in Figure 1. The detection of two different charge states for a fragment allows the assignment of charge and mass with greater certainty. This is particularly useful when the singly charged ion would be beyond the mass range of the instrument as in the TIQ fragment. Some results of a scanning experiment under the same conditions over the mass range from 710 to 1220 daltons at 1.3 sec/scan are shown in Figure 2. The spectra were taken as the TIQ fragment eluted. The doubly and triply charged ions are prominent. In addition, dissociation of the ions in the mass spectrometer interface produces ions characteristic of the sequence of the...
fragment. With good resolution in the separation, such fragmentation is equivalent to an MS/MS experiment and permits the unambiguous identification of the fragment in cases where the mass alone is insufficient.

This work was generously supported by Beckman Instruments Inc. and the U. S. Department of Energy, Office of Health and Environmental Research, under contract DE-AC06-76RLO 1830.

Pacific Northwest Laboratory is operated by Battelle Memorial Institute.


Figure 1. UV trace and single ion plots for two fragments from a tryptic digest.

Figure 2. Spectra from the T10 fragment showing two charge states of the parent and sequence specific fragments.
CAPILLARY ZONE ELECTROPHORESIS COMBINED WITH TANDEM MASS SPECTROMETRY

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INTRODUCTION

Although examples of CZE-MS have been reported, the structure information obtained other than the molecular weight is lean, and an MS/MS coupled to CZE is much more desirable.

GOAL

To couple coaxial flow FAB, developed by Tomer, Jorgenson and coworkers[1] with a tandem mass spectrometer having extended mass range array detection.

CONSIDERATIONS FOR CZE IN CZE/MS

- Column Diameter. Fine capillary(<13 um i.d.) has to be used on account of the vacuum of the MS source chamber. Loss of resolution and weak UV signals are resulted (Fig. 1).
- Column Length. The continuous FAB probe is ca. half a meter long; therefore, the capillary for CZE cannot be shorter than 0.8 m. Rapid analysis is not likely.
- Viscosity of the Buffer. We suggest that efforts to increase viscosity are not productive.
- Voltage. The highest available voltage 30 kV of the CZE unit is reduced to an effective voltage of 22--24 kV due to an accelearing voltage of 6--8 kV at the probe tip, leading to considerably lower potential gradient.

CONSIDERATIONS FOR THE CZE/MS INTERFACE

Coaxial Flow FAB.
1. The probe is composed of a long coaxial flow path (see Fig. 2) and band broadening is kept minimal.
2. Such a probe is compatible with low flow rate analysis system such as micro HPLC or CZE, the latter being especially suitable.

CONSIDERATIONS FOR THE MASS SPECTROMETER

Operating Parameters.
1. Scan Speed. The high scan speed of ZAB-T matches the sharp peaks of CZE.
2. Sensitivity. The array detector provide a high sensitivity, on the order of low femtomoles and meets the demanding sensitivity requirement of CZE with a fine capillary.

EXPERIMENTAL

1. CZE Apparatus. ISCO (Lincoln, NE) Model 3850.
2. MS. VG ZAB-T, (Manchester, England) of B-E-B-E four sector design and a 15 x 15 cm array detector.

RESULTS

1. Sensitivity of the MS. At a mass flow of 12 fmol/s of brakdykinin, the observed S/N for detecting the molecular ions is approximately 50:1.
2. Mass spectrum of bradykinin introduced through the CZE capillary. See Fig. 3.
3. Further details are to be studied.

Figure 1. Electropherograms of capillaries of different sizes. UV detection at 215 nm. (A) Capillary 50 μm i.d., total length 80 cm, effective length 60 cm, 0.005 AUFS applied field strength 275 V/cm, sample introduced by siphoning at a head of 14 cm for 7 s, injected amount: levitide 0.11 pmol, bradykinin 0.37 pmol, angiojenin 0.17 pmol, kassinin 0.28 pmol and DBI 0.18 pmol. (B) Capillary 10 μm i.d., total length 65 cm, effective length 45 cm, 0.01 AUFS, applied field strength 240 V/cm, sample electroinjected at 10 kV for 10 s, component amount: levitide 1.3 pmol, bradykinin 4.3 pmol, angiojenin 1.5 pmol, kassinin 1.9 pmol and DBI 1.2 pmol.

Figure 2. Schematic diagram of the flow FAB probe. (Courtesy of Drs. M. A. Moseley and K. B. Tomer).

Figure 3. Mass spectra of bradykinin
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ELECTROCHROMATOGRAPHY/ESI/MS USING NANOSCALE CAPILLARY LC

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Electrochromatography consists of liquid chromatography in which an electrical potential is applied across the column (1-4). This separation method, which utilizes mobility and sorptive interactions simultaneously, may be an alternative to CZE or conventional liquid chromatography for separation of components.

There are several potential advantages associated with electrochromatography. One advantage is the plug-glow obtained by electroosmotically driven chromatography which can result in higher efficiencies. The portioning of analytes between stationary and mobile phase is identical (3). Another advantage is the use of the high voltage as a modifier in conventional HPLC. In these experiments, a potential is used to retard or elute charged components (4). Verheij has also recently described the combination of electrochromatography with MS using CF-FAB interface.

The nanoscale electrochromatography column used in these experiments consists of a 135 cm long by 75 μ id fused silica column. The column is packed to a depth of 31 cm with Vydac AQC18. A short, 0.1 - 0.3 mm length of tubing that has been drawn out to a fine tip is used as the frit. The injection end of the column is placed in a buffer solution inside of a plexiglass pressure vessel. The high voltage source for the electrochromatography is wired into a lead that is inserted into the buffer solution inside the reservoir. Pressure for the LC component is provided by helium pressure introduced into the plexiglass vessel via a fused silica line. The entire apparatus is enclosed in a plexiglass box fitted with safety interlocks (the same as used for our CZE/MS interface). Samples are loaded onto the column using pressurized introduction. The column is inserted into the sheath flow column of a modified Vestec ESI probe. The end of the column is at the ESI needle, which acts as ground for the electrochromatographic column (3 kV). The mass spectrometer used is a VG 12-250 quadrupole mass spectrometer that has been fitted with a Vestec 611B Electro spray source.

As an example of electrochromatography the separation of the aminobenzoic acid ethyl ester (ABEE) derivatives of three oligosaccharides (2-acetamido-2-deoxy-6-O-(B-D-galactopyranosyl)-D-glucopyranose ABEE derivative, 2-acetamido-2-deoxy-3-O-(B-D-galactopyranosyl)-D-glucopyranose ABEE derivative, and 2-acetamido-6-O-(2-acetamido-2-deoxy-B-D-galactopyranosyl)–2-deoxy-D-glucopyranose ABEE derivative) is shown using nCLC, CZE and electrochromatography. Elution orders under EC are the same as with nCLC, but retention times are shorter.

These examples demonstrate the feasibility and the advantages of coupling electrochromatography with ESI/MS. Good separations and detection limits are observed. Separations are different from those obtainable by nCLC or CZE alone. This electrochromatography system is simple and can be used either as nCLC (without high voltage) or CZE (without pressure). The columns are quite easy to pack. Electrochromatography might be another way to solve separation problems in addition to CZE and LC.

References
5. Verheij, et al., 7th (Montreux) Symposium, Montreux, Switzerland, Oct., '90.

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nCLC / ESI / MS : ABEE SUGARS

Chromatograms from a mixture of amylobenzoic acid ethyl ester sugar derivatives
Column: 1.35 m x 75 μm, 43 cm packed with 10μ Hypersil C-8,
Mobile phase: 10% CH₃CN: 0.01 M ammonium acetate (0.001% TFA)

CZE / ESI / MS : ABEE SUGARS

Chromatograms from a mixture of amylobenzoic acid ethyl ester sugar derivatives
Column: 1.35 m x 75 μm, 43 cm packed with 10μ Hypersil C-8,
Mobile phase: 10% CH₃CN: 0.01 M ammonium acetate (0.001% TFA)

nanoscale ELECTROCHROMATOGRAPHY / ESI / MS : ABEE SUGARS

Chromatograms from a mixture of amylobenzoic acid ethyl ester sugar derivatives
Column: 1.35 m x 75 μm, 43 cm packed with 10μ Hypersil C-8,
Mobile phase: 10% CH₃CN: 0.01 M ammonium acetate (0.001% TFA)
The Determination of Veterinary Drugs using Nanoscale Separation Techniques In Combination with Electrospray Mass Spectrometry.

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Introduction

In animal husbandry and fish farming veterinary antibiotics may be administered to livestock for therapeutic purposes or to promote growth. Absorption of the drugs in tissues may occur such that when the half life of the drug is sufficiently long then there is a risk of the presence of residues in tissues prepared for human consumption. The low levels at which these drugs may be present in tissues mean that sensitive analytical methods are required for their screening.

Our laboratory has been interested in the applications of nanoscale capillary liquid chromatography (nCLC) using 2m x 75 μ l.d. packed capillary columns packed to a depth of 25cm with 10 μ AQ C-18 and capillary zone electrophoresis (CZE) using 75 μ l.d. fused silica capillaries in conjunction with electrospray (ESI) mass spectrometry. These techniques utilize sub-microliter flow rates so sample consumption is limited. The make up flow (50:50 methanol : 3% acetic acid ) required for stable operation of the electrospray source is provided through the electrospray probe via a co-axial arrangement. Water containing 0.1% TFA was solvent A throughout the nCLC work. Solvent B was acetonitrile containing 0.1% TFA for the sulfonamide and acid separations and methanol containing 0.1% TFA for the tetracyclines. The buffer employed for CZE was ammonium acetate adjusted to pH 7 with ammonium hydroxide. The mass spectrometer used throughout the experimental work was a VG 12-250 quadrupole with a Vestec electrospray source and controller.

Results and Discussion

Electrospray spectra have been successfully acquired for a variety of veterinary drugs including sulfonamides, quinolone carboxylic acids, tetracyclines, β lactams, lincosamides, aminoglycosides and macrolide antibiotics. At low skimmer voltages the spectra are dominated by protonated molecular ions except for the aminoglycosides which mainly showed (M+2H)+ ions. Increasing the skimmer voltage can be used to produce CAD type spectra. For the sulfonamides this was of particular interest because four pairs of isomers were amongst those under study. The fragmentation of the sulfonamides all followed the same pathways and it was found that for three of the pairs that little differentiation could be made on the basis of relative abundances of daughter ions. Sulfamethazine and sulfisomidine, however, showed some difference at skimmer voltages > 25 V where the ion of m/z = 124, due to the (RNH)3+ dominated the spectrum of sulfisomidine while sulfamethazine showed several ions of comparable relative abundance. The isomeric pair of oxolinic acid and flumequine in the quinolone carboxylic acid mixture showed markedly different spectra due to major structural differences. At higher voltages the daughters mainly resulted from loss of side chains. Fragmentation of the aromatic skeleton was only seen at 75 V. The tetracyclines showed evidence of consecutive losses of H2O and/or OH from the protonated molecular ion at low voltages (Fig 1). Increasing the skimmer voltage had no discernible effect other than the increase in relative abundance of these fragments relative to the (M+H)+ ion.

To date we have investigated the on-line coupling of nCLC and CZE with ESI/MS for the determination of standard mixtures sulfonamides, quinolone carboxylic acids and tetracyclines. The sensitivity of the technique was found to be compound dependent where 5 to 10 times the sample loading was required for the tetracyclines relative to the sulfonamides and the quinolone carboxylic acids. Using nCLC/ESI/MS the sulfonamides were readily detected at the 50 to 70 pmole level but at the 30 pmole level detection limits were approached for some of the less sensitive species. For a simple mixture of nine sulfonamides a rapid gradient from 0% B to 50% B in 30
minutes was utilized but for a more complex mixture of 19 this was slowed to one hour. Three of the four isomeric pairs of the sulfonamides could be readily resolved using these gradients. CZE could be used to resolve sulfamethoxypyridazine and sulfameter. CZE/ESI/MS provided a comparable separation of the mixture of 9 sulfonamides in a third of the time with an order of magnitude increase in sensitivity. The disadvantage of CZE was that the upper limit of loading of the column was close to the detection limit of the mass spectrometer so the dynamic range was small. At this level noise spikes in the single ion electropherograms also seemed relatively intense.

The quinolone carboxylic acids could be seen readily at the 10 to 20 pmole level by nCLC/ESI/MS. All four acids were baseline resolved with a gradient from 0% B to 100% B in 15 minutes. CZE was of interest for the quinolone carboxylic acid because both oxolinic and piromidic acids can yield tailing peaks using LC. The quinolone carboxylic acids could be detected readily at the 1 - 3 pmole level where the elution order of the acids was altered relative to nCLC.

A mixture of tetracyclines, oxytetracycline and chlortetracycline was studied by nCLC where their structures only differ by one substituent group at either C6 or C7. They were readily resolved when a gradient from 0%B to 100 %B in 30 minutes was employed (Fig 2). This corresponds to an injection of 170 to 330 pmoles on column.

In our opinion nCLC/ESI/MS and CZE/ESI/MS are complementary techniques and can readily be applied to the determination of veterinary antibiotics.

Electrospray (ESI) and fast atom bombardment (FAB) are the "soft" ionization methods of choice for the mass spectral analysis of polar and thermally labile compounds. A major problem with these soft ionization techniques is that their ionization is dependent on the other components present in the matrix being analyzed. This discrimination may diminish the response or even prevent the compound from being observed. The use of liquid chromatography (LC) eliminates many of these problems in addition to providing a time domain to distinguish analyte and background. The use of packed capillary LC to interface to either continuous flow FAB (CF-FAB) or ESI is the preferred technique in both cases. The same system can be used for both techniques with minor modifications. The use of large sample injections combined with gradient LC allow chromatographic integrity to be maintained. Combined with the low flow rates (approx. 3 ul/min) of packed capillary LC columns, this allows the entire effluent to be directed to either MS technique with high sensitivity in the very low picomole range.

CF-FAB data were acquired on a VG ZAB 2-SE system while the ESI data were acquired on a Finnigan MAT TSQ-700 system. The capillary columns (320 um i.d., fused silica) were fabricated in-house.

The two mass spectrometric techniques often provide complimentary information dependent on the sample types. In the case of peptides, as others have reported for semi-micro LC, CF-FAB often provides sequence specific fragmentation while electrospray can cover a larger mass range due to multiple charging. This is illustrated for the tryptic digest of ubiquitin for which incomplete digestion occurs. For the T3 tryptic fragment, ESI gives only the singly and doubly charged protonated molecular ion species while the FAB mass spectrum contains significant fragmentation which allows a majority of the fragment's sequence to be determined as illustrated in Figure 1. The ESI spectrum of the partially digested ubiquitin shows two multiply charged ion series. Deconvolution gives the molecular weight for losses at the C-terminus of ubiquitin consisting of GG (small) and LRGG (large). This is shown in Figure 2.

For profiling of urinary metabolites (sulfates and glucuronides) of a smaller molecule, MDL 11,939, valuable information is obtained by both techniques. Again, the fragmentation observed in FAB can provide structural information lacking in ESI without the use of MS/MS. For larger molecules such as the glycopeptide teicoplanin, FAB and ESI both give only molecular weight information. However, some less hydrophilic trace components were observed by ESI and not by FAB. In conclusion, capillary LC interfaced to either technique is an excellent method for increasing sensitivity and reducing matrix effects for either FAB or ESI. For peptides below 2000 daltons, FAB offers sequence specific fragmentation while ESI can give spectra of large peptides and proteins with great sensitivity. Work on evaluation of both techniques with a variety of compound classes will help define the complimentary role of FAB and ESI as LC-MS techniques.
Figure 1. Analysis of tryptic digest of ubiquitin by LC-FAB/MS. FAB mass spectrum and RIC for T3 fragment (TITLEVEPSDTIENVK).

Figure 2. Analysis of tryptic digest of ubiquitin by LC-ESI/MS. ESI mass spectra and RIC for T3 fragment. Also mass spectrum (conventional and deconvoluted) for partially digested ubiquitin.
Rapid Identification of Microcystins in the Cyanobacteria by Frit-FAB LC/MS

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Introduction
Several genera of fresh and brackish water cyanobacteria, Microcystis, Oscillatoria, Anabaena, Aphanizomenon and Nodularia, produce some kinds of acute lethal toxins, and deaths of wildlife and domestic animals have been ascribed to taking toxin-containing water. Toxins from these genera are known to include cyclic hepatotoxic hepta- and pentapeptides and neurotoxic alkaloids. Cyclic hepatotoxic heptapeptides named microcystins consist of a common moiety composed of five amino acids, 3-amino-9-methoxy-10-phenyl-2,6,8-trimethyldeca-4,6-dienoic acid (Adda), N-methyldehydroalamine (Mdha), D-alanine, \( \beta \)-linked D-erythro-\( \beta \)-methylaspartic acid, and 7-linked D-glutamic acid, and two L-amino acid variants, and thirteen microcystins have been isolated so far (Fig. 1). Three new cyclic heptapeptides were recently isolated from Nostoc species, which have an acetyl group in Adda moiety instead of a methoxy group in usual microcystins. Lately it has been demonstrated that microcystins inhibit strongly protein phosphatases 1 and 2A. In this study we developed an analysis method using Frit-FAB LC/MS for rapid separation and identification of microcystins.

Experimental
A mass spectrometer (JMS-AX505W, JEOL) was used and connected to a data system JEOL JMA-DA5000. A JEOL Frit-FAB Probe with a stainless steel frit was connected to a pneumatic splitter. The FAB mass spectra were obtained in positive-ion mode by scanning from mass 50 to 1500 with a scan speed of 8 s/cycle. A FAB gun was operated at 3 kV with xenon gas. Injected samples were introduced into ion source at a split ratio of 8/500 after post-column splitting with the pneumatic splitter. A high performance liquid chromatograph equipped with a constant-flow pump (LC100 system, Yokogawa) was used. Separation was accomplished under reversed phase isocratic conditions on an ODS column (Nucleosil 5C18, 150 x 4.6 mm) with mobile phases of (a) MeOH:0.01% TFA (55:45) (containing 0.8% glycerol) and (b) MeOH:0.1M AcONH\(_4\) (adj. pH 5 with TFA) (53:47) (containing 0.8% glycerol). The flow rate was 0.5 ml/min and UV absorbance at 238 nm was used as the detection method.

Results and Discussion
FABMS and liquid SIMS have been only useful technique for determination of molecular weights of microcystins because it was very difficult to ionize these intact toxins by other ionization methods. Although several LC/MS interfaces, Frit-FAB, thermospray, atmospheric pressure ionization, continuous flow FAB, have been developed, Frit-FAB LC/MS was considered to be promising for our purpose. In our previous reports on HPLC for separation of microcystins, the following three solvent systems have been complementarily used: MeOH:0.05M phosphate buffer (pH 3)=6:4, MeOH:0.5M Na\(_2\)SO\(_4\)=1:1 and MeOH:0.05% TFA=6:4. TFA-containing solvent system was considered to be useful, because acidic matrix is suitable for ionization of microcystins. So the Frit-FAB LC/MS using the TFA-containing mobile phase could be successfully applied.

The HPLC conditions slightly modified, MeOH:0.01% TFA=55:45 (containing 0.8% glycerol) as a mobile phase and 0.5 ml/min as a flow rate, provided a base line separation of standard microcystins RR, YR and LR, and their geometrical isomers (RR-s, YR-s and LR-s) (Fig. 2). In this system a portion of 8/500 of the sample was introduced into ion source of mass spectrometer. The remaining part of the effluent was introduced into an usual UV (238 nm) detector for HPLC via a pneumatic splitter and the high performance liquid chromatogram was simultaneously measured. If larger amounts of samples are loaded, the preparative separation is also available using this system. Mass chromatography is a useful technique for identification of a desired compound, when its molecular weight is known. In fact, the technique enabled a rapid identification of the six components as shown in Fig. 2. Sixteen microcystins have been reported so far, whose molecular weights are different one another except for the case of 3- and 7-desmethylmicrocystins LR. The use of mass chromatography makes possible a rapid identification of at least fourteen microcystins. Additionally, both desmethylated microcystins LR were separated and identified by another Frit-FAB LC/MS using the mobile phase (b).

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The established method was subsequently applied to analysis of microcystins contained in both a culture strain and a field sample, and the procedure from toxin extraction to identification of microcystins was performed within one day. These results allowed the rapid identification of known microcystins without standard samples. In addition, other types of compound than microcystins were also detected in the course of screening of various samples by the method.

References

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* L-aminooxybutyric acid

Fig. 1

![Diagram of microcystin structure](image)

Fig. 2

![Mass spectrum of microcystin RR](image)
COMBINATION OF TEMPERATURE-PROGRAMMED CAPILLARY LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRY

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LC/MS with solvent gradient programming has become a powerful technique for the separation and identification of complex unknown samples of biochemical significance. However, the efficiency of most LC/MS interfaces based on vaporization, nebulization or desolvation processes varies during gradient programming because of the change in viscosity and surface tension of the mobile phase (1, 2). The use of temperature programming with isocratic mobile phase composition is an alternative solution to this problem. Temperature-programmed LC/MS may be achieved by using packed capillary columns, which have high separation efficiency and relatively modest demands for instrumentation (3). The narrow diameter (100-300 μm) and low flow rate (0.1-10 μL/min) ensure rapid heat transfer across the column during temperature programming and require only a simple LC/MS interface for the introduction of analyte under EI conditions. Application of temperature programming to capillary LC/MS provides a reduction in total analysis time with optimization of chromatographic resolution and an improvement in detection limits for highly retained solutes.

In this study, the temperature-programmed capillary LC/MS system shown schematically in Figure 1 has been developed. A single-piston reciprocating pump was used in the constant-flow mode to deliver the mobile phase. A conventional 1 μL injection valve was used for sample introduction, and the effluent was subsequently split to provide a flow rate less than 1 μL/min to the packed capillary column. A 200 μm I.D. x 40 cm column was prepared from 5 μm spherical silica particles with an octadecyl stationary phase. Both the column and splitter were kept in an oven to maintain a constant split ratio during temperature programming. A standard GC oven was modified for programming rates from 0.1 to 0.5 °C/min between 40 and 100 °C in order to be compatible with liquid chromatographic conditions. The effluent from the column flows sequentially through a UV absorbance detector, the LC/MS interface, and into the MS ion source. A direct coupling LC/MS interface with thermocouple and heater was designed to control the interface temperature independently. This interface can be inserted through a vacuum manifold for the direct insertion probe of a HP 5985 MS. The quadrupole MS was operated in the EI mode at 70 eV while scanning the mass range from m/z 50 to 400 in 4 to 5 seconds.

The performance of the capillary LC/MS interface was evaluated with a test mixture containing phenol, nitrobenzene, toluene, anthracene, and pyrene. The mixture was separated on the reversed-phase column using acetonitrile as mobile phase and the individual components (500 ng each) were identified by their EI mass spectra (Figure 2). To study the effect of interface temperature on the evaporation of nonvolatile solutes, a square wave pulse of the solute was introduced directly into the ion source. At 180 °C, sporadic evaporation of the solute was observed due to insufficient heat. As the temperature was increased, both peak shape and signal to noise ratio were improved. The optimum interface temperature was between 250 °C and 270 °C. Repeated injections showed 11% relative standard deviation in peak height from reconstructed mass chromatograms using individual fragment ions or total ion current.

The temperature-programmed capillary LC/MS system was applied to the separation and identification of unknown fatty acid constituents in a dietary fish oil sample. Figure 3 permits a comparison of chromatograms based on UV detection (top) and reconstructed mass chromatograms (bottom). Under optimized conditions, the separation of twelve methyl esters of fatty acids was achieved with baseline resolution of the reversed-phase column with 75% aqueous acetonitrile at a temperature programming rate of 0.34 °C/min from 40 to 80 °C. The saturated and unsaturated esters with fewer than two double bonds provided poor sensitivity with UV absorbance detection due to their low molar absorptivity. However, the reconstructed mass chromatograms showed a response for saturated and unsaturated esters with higher sensitivity. The C\(_{5}\)H\(_{11}\) ion at m/z 55 was used for universal detection of both saturated and unsaturated esters. The ions at m/z 74 (from the McLafferty rearrangement), 75, 87, and 143 were characteristic fragment ions from both saturated and mono-unsaturated esters, while the C\(_{5}\)H\(_{11}\) ion at m/z 96 was selective for mono-unsaturated esters. The C\(_{5}\)H\(_{13}\) ion at m/z 77, C\(_{5}\)H\(_{17}\) ion at m/z 79, and C\(_{5}\)H\(_{19}\) ion at m/z 91 were characteristic fragment ions from the highly unsaturated esters with more than three double bonds. The C\(_{5}\)H\(_{11}\) ion at m/z 91 from docosahexaenoate (22:6) was verified by collision induced dissociation with dimethyl ether using a Finnigan TSQ-70B triple quadrupole MS. The daughter ion mass spectrum of the parent ion at m/z 91 is identical to that of the tropylium ion from toluene as shown in Figure 4. The ion/molecule reaction of the tropylium ion with dimethyl ether produces collisional complexes consisting of the C\(_{5}\)H\(_{11}\)-CH\(_{3}\) ion at m/z 105 and the C\(_{5}\)H\(_{17}\)-CH\(_{3}\)OCH\(_{3}\) ion at m/z 137. These results strongly suggest that the peak at m/z 91 in the mass spectrum represents the tropylium ion as the isomeric tolyl ion also forms the C\(_{5}\)H\(_{14}\)(CH\(_{3}\))OCH\(_{3}\) ion at m/z 122 upon reaction with dimethyl ether (4), and no peak at m/z 122 was observed in the present work.

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Fig. 1. Schematic diagram of temperature-programmed capillary LC/MS system.

Fig. 2. Reconstructed mass chromatograms for the separation of aromatic test mixture (left) and their corresponding EI mass spectra (right).

Fig. 3. UV absorbance (top) and reconstructed mass chromatograms (bottom) for the detection of fatty acid constituents in a commercial fish oil product separated by temperature-programmed capillary LC.

Fig. 4. The CID daughter ion mass spectrum of C_{27}H_{48}^+ (m/z 91) from toluene (bottom) and docosahexaenoate (top) in reaction with dimethyl ether.

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Ultrasound Based Direct Liquid Introduction CI Mass Spectrometry

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A simple, easily fabricated interface for flow injection and micro scale LC/MS has been developed which is based on a high frequency low capacity ultrasonic nebulizer. Additional nebulization and solvent declustering is provided by 2 to 5 mL/min of helium gas which flows coaxially around a 50 μm i.d. fused silica capillary which is used to admit sample directly into the source of a Finnigan 4023 quadrupole mass spectrometer. This design avoids the plugging and fabrication problems associated with the 5 μm or smaller constrictions which have been used with purely pressure driven systems. "Tight" source conditions were maintained with the aid of a polyimide foam gasket placed between the nozzle barrel and the source block. Conventional positive and negative CI spectra have been generated for common test solutes, e.g. caffeine and C17 fatty acid, as well as more difficult compounds. A high abundance molecular ion was generated under NCI conditions for an unknown macrolide which failed to yield unambiguous molecular weight information with direct probe methods or static FAB.

We are currently attempting to miniaturize the prototype interface such that it can be used with existing 1/2 inch direct probe inlets. Presently the interface requires a separate bolt on flange due to the size of the nozzle. With the recent advent of commercially produced 3/8 inch dia. ultrasonic nebulizers, which resist detuning at the temperatures encountered in MS work, such miniaturization is a realistic goal.

Our device differs from that reported by Ligon and Dorn (1) in that it was optimized for the low liquid flow rates, < 10 uL/min, required of DLI interfaces and CFLOW (Continuous Flow) FAB MS. It has generated steady sprays at flow rates down to 100 nL/min. Ligon and Dorn used a similar commercially available ultrasonic nozzle as part of their design for a particle beam type interface, a momentum separator designed to operate at flow rates on the order of 2 or 3 mL/min. Although Games reported the existence of German and Japanese patents for ultrasonically based LC-MS interfaces in a 1983 review (2), reports in the open literature of successful application have, to our knowledge, been limited to the work described here and that referenced above (1).

The combination of high frequency (120 kHz) ultrasound and gas nebulization has been observed to have desirable properties, applicable to many different types of LC-MS interfaces in addition to the specific device described here. These include overall mechanical robustness, ease of construction, and the absence of critical adjustments and orifices.

References


Above: exploded view of the helium assisted ultrasound based DLI Interface. All parts were fabricated from 316 stainless steel unless indicated otherwise. The fused silica capillary was connected to an HPLC injector and a switching valve which formed part of a helium pressure driven flow injection system.

Below: DLI negative chemical ionization mass spectrum of an unknown whose structure has been partially determined. NMR, IR and elemental analysis are consistent with the molecular weights indicated.

**Unknown macrolide**

\[ R_3 = \text{H} \quad \text{free alcohol} \quad C_{22}H_{48}O_9 \quad \text{MW 428} \]

\[ R_3 = \text{-} \quad \text{acetate} \quad C_{24}H_{28}O_{10} \quad \text{MW 468} \]

NCI conditions, DLI
USE OF THE MOVING BELT INTERFACE FOR ON-LINE CHARACTERIZATION OF HIGH MOLECULAR WEIGHT POLYCYCLIC AROMATIC COMPOUNDS IN ENVIRONMENTAL SAMPLES by LC/MS and SFC/MS

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One of the most powerful techniques for characterization of Polycyclic Aromatic Compounds (PAC) in environmental matrices is the combination of HPLC with mass spectrometry. The use of HPLC/MS interfaces based on mechanical transfer devices, such as a moving wire or moving belt, has fallen into disfavour in recent years, due mainly to the popularity of thermospray, particle beam, electrospray and ion spray interfaces. These newer techniques have largely supplanted the mechanical transport devices.

However, in our hands the moving belt has proven to be a reliable and efficient HPLC/MS interface for thermally stable analytes of moderate volatility, especially PAC. The ease of compatibility of this interface with EI and CI ionization sources makes it very suitable for the analysis of these compounds. The moving belt has also been shown to be a suitable interface for packed column SFC/MS.

Most applications of this interface to the HPLC analysis of PAC have been limited to compounds of molecular weights not much higher than the range accessible by normal GC/MS. Developments of HPLC methods based on UV and/or fluorescence detection on the other hand have involved the separation of PAC's of molecular mass up to 600 Da. We report here on the application of the moving belt interface to the on-line monitoring of the PAC in a complex coal tar sample, for compounds of molecular mass up to 580 Da. Also reported here are some preliminary results from a normal phase SFC/MS method, developed using the moving belt as the interface. It is hoped to adapt this SFC method to a preparative fractionation technique.

The sample studied here was obtained by extracting and fractionating excavated bulk material from the Sydney Tar Pond, Sydney, Nova Scotia. This site is the result of 60 years of uncontrolled discharge of effluent from a metallurgical coking operation. The sample was first analysed by reverse phase HPLC/MS. The solvent gradient system was a modification of that employed earlier for analysis of a carbon black extract. Later work involved SFC/MS analysis. Carbon dioxide, often used as a mobile phase in SFC, would not elute high molecular weight PAH within reasonable analysis times. We found pentane to be a suitable solvent for this purpose; with the addition of methanol as a modifier, it provides reasonable normal phase separation of PAC on a packed silica column. The moving belt interface was very suitable for this SFC/MS work.

Typical HPLC reconstructed ion chromatograms, for m/z 350 and 578, are shown in Figure 1. The belt transmits eluted compounds to the mass spectrometer (EI mode) with good preservation of chromatographic peak integrity. The column performance, in terms of isomer separation, appears to be better for the cata-condensed than for peri-condensed systems, as a general trend. This feature is almost certainly a manifestation of the well known molecular shape selectivity of polymeric phase columns.

In the mass spectra shown in Figure 2, the compounds depicted are examples of the possible PAH of the indicated masses. Work is proceeding on identification of the actual compounds present but is hampered by the lack of suitable standards. The ability of the belt interface to permit acquisition of good quality mass spectra, even for larger compounds, is evident from Figure 2. These spectra are like those of smaller PAH, in that they are dominated by molecular ions. The background from the belt is relatively unimportant at these higher masses.

Figure 3 shows the total ion chromatogram obtained from an SFC/MS normal phase analysis of the Tar Pond sample. Each fraction of a given molecular weight range may be collected and analysed off-line by HPLC/MS techniques.
The HPLC/MS and SFC/MS techniques described here offer a combination of retention and molecular weight data which can be invaluable in characterizing complex samples.

Figure 1: Reconstructed HPLC/MS ions chromatograms. a) m/z 350, b) m/z 578

Figure 2: Mass spectra obtained by HPLC/MS (EI, 70 eV) a) Molecular ion at mass 350; b) mass 578. Compounds depicted are possible PAH of the corresponding mass.

Figure 3: Total ion chromatogram obtained by SFC normal phase separation of the Tar Pond sample. Numbers indicate masses of eluting PAC.

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Federal and State mandates for groundwater and soil dissipation studies usually require monitoring of pesticides used at low rates down to the part per billion level in soil and the sub-part per billion level in water. In addition to the soil and water monitoring studies, if the pesticide is to be applied in the field through aerial application, pesticide drift studies may be required in order to monitor off-target movement.

Migration of the pesticide during and after the aerial application is monitored by a combination of passive and active collection devices placed throughout the field and the downwind area which collect the sprayed material. Analysis of the filter pads then leads to a mapping of the spray drift which can be used in conjunction with application and weather data to characterize the off-target area.

Sulfonylureas, including primsulfuron-methyl, are a major group of commercially important herbicides which are very active at very low use rates, corresponding to subsequent low residue concentrations. In addition, many of these compounds readily decompose under normal environmental conditions. Because of the low use rates of sulfonylureas and their thermally labile nature, sensitive and reliable analytical methods are needed for their analysis. High performance liquid chromatography coupled with mass spectrometry (HPLC/MS) provides the needed selectivity and sensitivity.

Furthermore, by using the high resolution capabilities of a double focusing mass spectrometer, not only can many of the background interferences be easily eliminated but the level of confidence is increased by the enhanced specificity of the ion selection process. Because of the reduced background, extraneous clean-up steps during the extraction are not necessarily needed, thus contributing to a relatively minimal sample preparation procedure. The simple preparation procedure also helps avoid additional sample losses which lower sample recovery during extraction.

We have developed a simple extraction procedure requiring sonication of the filter pads (collected from spray drift studies on primsulfuron-methyl) in methanol followed by concentration using Kuderna-Danish techniques. The final concentrated extracts are then injected onto a normal phase HPLC column, eluted with 30% 1:1 methanol/isopropanol in cyclohexane and analyzed by single ion monitoring mass spectrometry using the moving belt interface. The mass spectrometer was operated using electron impact ionization and medium resolution (5000-6000). Mass 187, which is the most intense ion in the electron impact spectrum of primsulfuron-methyl, was used as the quantification ion and mass 199 was used as the confirmation ion.

Figure 1 shows the single ion chromatograms, at different instrument resolutions, of a sample extract obtained from a filter paper which was placed on the field during the aerial application of primsulfuron-methyl. Figure 2 represents the chromatograms of 10 ul of the lowest (0.1 ppm) calibration standard used for the study obtained under similar resolution conditions as the sample extract. Although the sample extract is sufficiently concentrated to be observed reliably at a resolution of 500 (Fig. 1a), the lowest standard (Fig. 2a) cannot be detected with any degree of confidence at the same resolution. At a resolution of 6000, however, the background interferences for the same sample extract (Fig. 1c) are completely eliminated and the lowest calibration standard (Fig. 2c) is easily recognizable. Moreover, the peak which is
obtained and quantified at 6000 resolution can be attributed to primsulfuron-methyl with a greater
degree of confidence than the corresponding peak at 500 resolution.

Using this analytical procedure the limit of quantification of primsulfuron-methyl extracted
from filter pads is 1.0 ng on column, representing twice the limit of detection. Moreover, this
approach can be used to quantify primsulfuron-methyl or other related compounds at similar
detection levels from matrices such as well-water and soil which have many more interferences
and require additional and sometimes complicated clean-up procedures.

Figure 1 HPLC/MS single ion chromatogram of m/z 187 from filter paper extract obtained at resolution of a) 500
b) 2000 and c) 6000.

Figure 2 HPLC/MS single ion chromatogram of m/z 187 from 0.1 ppm (1.0 ng on-column) primsulfuron-methyl
standard obtained at a resolution of a) 500 b) 2000 and c) 6000.
Development and Refinement of an Element and Isotope Selective Detector for HPLC Based on Mass Spectrometry.

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Introduction:

The CRIMS (Chemical Reaction Interface for Mass Spectrometry) technique was first developed by Markey and Abramson in 1982 as a creative way of utilizing the mass spectrometer as an element and isotope selective detector for gas chromatography. ¹ The basic principle is to feed the chromatographic effluent into a microwave powered reaction cell where the molecular species are broken down to their elemental form. The addition of a reactant gas to the microwave plasma results in formation of small gaseous reaction products that are characteristic of the elements contained in the original molecule. Conventional EI mass spectrometry is then used to detect specific elements or isotopes in the form of these reaction products. For example, ¹⁴N labeled species are detected at m/z 31 as ¹⁴NNO and, analogously, ¹³C containing compounds are detected at m/z 43 as the combustion product ¹³CO₂. Structural information on the molecular species may be obtained by simply turning off the microwave power supply and allowing the intact molecule to enter the mass spectrometer's ion source.

The success of this method as a specific detector for gas chromatography, has led us to couple the CRIMS device to HPLC. Initial results presented previously² were very promising but in some cases suffered from poor signal-to-noise. The work reported here is an extension of this earlier work and concentrates on efforts to increase the signal-to-noise ratio for ¹⁴N, ¹⁵N, ¹²C and ¹³C detection by: 1) using high purity solvents which exhibit a low residual after evaporation, 2) eliminating mechanical pump backstreaming by adding in-line filters to the momentum separator pump-out lines, 3) improving sample transmission through our particle beam/CRIMS interface by utilizing a more compact interface design and, 4) using a specially designed EI/CI particle beam ion source for improved sensitivity.

Results:

The objective of this paper is to report the refinements made to the LC-CRIMS system, as stated above, and to demonstrate the utility of combining CRIMS with liquid chromatography to selectively detect and identify urinary metabolites of ¹⁵N₂, ¹³C labelled caffeine. The analysis of caffeine metabolites serves as an example of how the isotopic and element specific detection capability of CRIMS can aid in identifying drug metabolites contained in a complex matrix. A general procedure for identifying and confirming the structure of a drug metabolite using CRIMS and particle beam LC/MS is outlined in the following steps;

1) administer stable isotope labeled drug to the desired subject,
2) collect appropriate biological fluid,
3) perform routine sample work-up procedure,
4) obtain selective isotope chromatogram on the sample using the HPLC-CRIMS technique by monitoring m/z 31 (¹⁵NNO) for a ¹⁵N enriched drug or m/z 45 (¹⁵CO₂) for a ¹³C labeled drug, and lastly,
5) in a subsequent HPLC run, obtain an EI mass spectrum of the intact molecule at the retention time predetermined by the isotope selective chromatogram.

This procedure was followed in our laboratory to identify ¹⁵N₂, ¹³C-caffeine metabolites in dog urine with minimal sample clean-up (ie. filtration through a 0.22 micron filter with no further purification). An isotope selective chromatogram obtained from this urine sample is shown in figure 1. The equation at the top left-hand corner of this trace is the equation used for subtracting out the signal at m/z 31 (¹⁵NNO) due to naturally occurring ¹⁵N in the sample. Distinct peaks corresponding to ¹⁵N isotopically enriched caffeine metabolites are observed well above the baseline noise. In figure 2, the ¹⁵N NO response (panel B) is compared to the m/z 44 response which is representative of the total ion chromatogram obtained for this sample and also for other nonspecific detection methods. With nonspecific detection the caffeine metabolites are hidden by interfering components, but by monitoring the isotope specific product produced by the CRIMS technique, it is possible to extract the metabolite peaks and assign appropriate retention times for subsequent full scan particle beam EI LC/MS experiments.
Conclusion:

The CRIMS technique coupled to HPLC allows element and isotope selective detection of isotopically labeled, or intrinsically labeled, nonvolatile compounds to be realized. This appears to be particularly important in drug metabolism studies where retention times of metabolites can be determined even in extremely complex matrices. Once the precise retention time is known, full scan particle beam EI LC/MS experiments can be used to identify the metabolite through molecular weight information or structurally significant fragmentation. This methodology was successfully used in this study to identify caffeine metabolites in dog urine.

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The Interpretation of Daughter Ions from Multiply Charged Parent Ions
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The increasingly widespread use of liquid phase ionization techniques based on ion evaporation (ionSpray and Electrospray) has shown that the mass range for detectable molecules can be dramatically extended because of the ready formation of multiply charged ions. We have shown, for example, spectra obtained on a quadrupole mass spectrometer with a mass range of 2400 amu that allowed the molecular weight of immunoglobulin at mass 148,000 dalton to be determined.

There have been several reports indicating that multiply charged ions can be fragmented in a triple quadrupole and that this process generally requires less energy than does fragmentation of the corresponding singly charged species. Our efforts have mainly concentrated on fragmenting doubly charged tryptic peptide ions; these spectra are straightforward to interpret since the charges tend to be localized on opposite ends of the peptide backbone (the N-terminus and basic C-terminal lysine or arginine) and hence breaking any backbone bond leads to the formation of two singly charged daughter ions. Smith and co-workers [1,2] have reported on the fragmentation of intact proteins with much higher charge states and, based on prior knowledge of the sequence, have been able to assign structures to many of the observed ions. Clearly it would be advantageous to be able to interpret the daughter spectra obtained from multiply charged ions and, hopefully, to be able to extend the mass range for daughters in the same way as for intact molecules.

Experiments with model compounds, the small proteins renin substrate (MW 1758.9) and mellitin (MW 2845.8), show that there are data processing methods for obtaining useful data from the daughter spectra of multiply charged ions. Two phenomena are fundamental to these approaches:

1) Smith et al have observed on several occasions that the daughter spectra of multiply charged protein ions tend to show similar mass fragments in different charge states. This situation is analogous to a 'conventional' multiply charged spectra where mathematical relationships can be used to determine the molecular weight. In the case of daughter ions, the mass determined is that of the parent and the difference is that a series of multiply charged daughters may not differ by the addition of protons; the actual relationship depends on the fragmentation mechanism. The phenomenon is believed to arise from the heterogeneity of the multiply charged parent, i.e. that the an ion with with, say, 5 charges exists in several forms with the charges located at different sites in the molecule. Simple cleavage of backbone bonds will therefore tend to produce fragments with the same mass but a distribution of charges.

2) In any fragmentation the charge and mass must be conserved. If a parent produces two ions, rather than ion(s) and neutral(s), the sum of the masses and charges of the fragments must equal those of the parent. If the charge state of an observed ion is presumed then the corresponding mass and charge of the matching fragment can be calculated: the presence of an ion at the calculated mass/charge corroborates the initial presumption. This allows the charge state of some ions to be verified. In the case of proteins, such matching pairs are also sequence ions since they correspond to backbone fragmentation.
Processing daughter ion data using algorithms designed for obtaining the molecular weight from the spectra of multiply charged ions often reveals data that were not previously apparent. The spectra below illustrate the process of reconstructing a daughter spectrum and applying a 'hit' filter of 2. The hit filter eliminates ions having fewer contributions than the specified value. The processed data reveals the majority of the sequence ions and eliminates ions, for example the doubly and triply charged sequence ions, that add complexity to the original spectrum.

The major disadvantage of this approach arises from a low level noise continuum that causes artificial hits. The 'noise' is made up from unresolved peaks caused by the poor resolution obtained for multiply charged daughters due to a large kinetic energy release, and the presence of ions that come closer together in m/z as the charge number increases. More sophisticated methods of thresholding and peak location could avoid some of these difficulties.

Recent developments in mass spectrometry are dramatically expanding its capability for the analysis of biopolymers including proteins and peptides. However, the extraction of the large amounts of information embedded in the mass spectrometric data is complicated. Therefore, there has developed a need for specific computer software for the convenient manipulation, interpretation, and display of the unique types of data generated in the analysis of these molecules. We have developed a suite of computer software to assist protein and/or peptide structure analysis by mass spectrometry.

**Molecular Weight Determination for Biopolymers -- BIOMASS Deconvolution Program:**
To facilitate the interpretation of ESI/MS spectra with multiple-charged ion distribution, we developed a deconvolution algorithm (1) which overcame the interference of "side peaks" from the algorithm described previously (2). This algorithm transforms the coherent series of multiply charged ions into a mass scale spectrum free of artifactual peaks, from which the parent mass can be readily determined. This allows the direct analysis of components of mixtures which would otherwise be difficult.

**Rapid Peptide Mapping of Proteins -- PEPMAP LC/MS Interpretation Program:**
Enzymatic peptide mapping (or finger-printing) with HPLC is one of the techniques most commonly used by protein chemists. Electrospray LC/MS is a very powerful tool for this purpose since it provides not only retention time but also structural information, namely the molecular masses, for every peptide in a digest. Nevertheless, the identification and matching of chromatographic peaks to every digested fragment from a known protein is still a tedious and formidable task. To speed up such efforts, we have developed an automatic program which allows rapid assignment of peaks corresponding to all types of predicted fragments, including complete, partial or incomplete, and disulfide-linked peptide fragments. The following shows the matching result of tryptic fragments of hemoglobin α-chain with LC/ESI/MS chromatogram for mixtures of tryptic digest of α and β chains:
All the matched fragments are automatically labeled with their scan numbers in the chromatogram. Similarly, tryptic fragments from β-chain can also be used to match with the same chromatogram and more peaks are identified by the program in this manner:

Additionally, some of the small peaks (•) in the chromatogram are identified to be the result of autolysis of the enzyme (trypsin) itself. Of those chromatographic peaks which cannot be accounted for in this first pass, other program tools are provided to assist further investigation for possible unanticipated cleavage or post-translational modifications.

Unknown Peptide Sequence Elucidation - PEPSEQ Sequencing Program:

Although the triple quadrupole mass spectrometer has been proven to be a powerful tool for peptide primary structure determination (3), interpretation of the resulting mass spectra can be a severe bottleneck to rapid analysis. A computer program has been developed to aid in the interpretation of low energy MS/MS mass spectra of unknown peptides using FAB MS/MS data in automatic, semi-automatic or manual mode (4). This program was successfully used to obtain the sequence information for the Protein Society workshop test peptide last year. A similar program is being developed to interpret ESI MS/MS data. A typical computer output of the interpretation of the CID spectrum for a doubly-charged parent of peptide FSWGAEGQR is showing here:

The interpreted result can be interactively matched by another program PEPMATCH which automatically labeled the spectrum with calculated sequence ions of any candidate peptide for rapid visual conformation. The preliminary results of the ESI/MS/MS sequencing program look promising, especially for tryptic peptides of up to 10-15 amino acid residues.

References:

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Manual or automated Edman degradation is the most widely employed method for N-terminal sequencing of peptides and proteins. Normally the sequentially liberated PTH-amino acids are identified by comparison with standard amino acid derivatives using HPLC separation.

Manual Edman degradation may alternatively be combined with mass spectrometric detection of the sequentially truncated rest-peptides/proteins. Both Field Desorption MS (1) and Fast Atom Bombardment MS (2) have been used for analysis of smaller peptides, and recently Plasma Desorption MS (3) have been employed. Advantages of this combination are primarily (i) the possibility of simultaneous sequence analysis of multi-component mixtures and (ii) the direct identification of posttranslational modifications.

The use of Plasma Desorption MS (PDMS) has been shown to be applicable to larger polypeptides (up to 15,000 Da (3)) and brings the additional advantage of the nitrocellulose interface, which makes it possible to employ a time-saving version of manual Edman degradation. The extraction- and washing-steps normally carried out in manual Edman degradation can be replaced by an "in-situ" rinsing step following sample application to the nitrocellulose surface. This step will remove most of the low-mass contaminants including excess coupling-reagent and liberated amino-acid derivatives, which otherwise may seriously hamper the molecular ion signal.

We have previously reported model-studies and several analytical applications of the combination of manual Edman degradation and PDMS (3). The technique has been further refined especially regarding optimization of PITC-coupling and sample application steps. Table 1 lists the obtained results from N-terminal sequencing of intact bovine insulin using the improved conditions. This analysis allows a direct identification of the intermolecular disulfide-bond between the two Cys\textsuperscript{7}-residues in the A- and B-chain, which is identified by the mass loss of 202.1 amu in the 7. cleavage-step. The Cys\textsuperscript{5}-residue, which forms an intramolecular disulfide-bond to Cys\textsuperscript{11} in the A-chain remains bonded to the rest-peptide following the 6. cleavage and would not be detected until the 11. cleavage step.
TABLE 1. N-terminal sequencing of intact bovine Insulin (MW 5733) using a combination of manual Edman degradation and PDMS.

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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<td>4981</td>
<td>4716</td>
<td>4735</td>
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<td>212.5</td>
<td>213.6</td>
<td>256.8</td>
<td>265.3</td>
<td>113.8</td>
<td>202.1</td>
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<td>Asn, Val, Gln, Glt</td>
<td>His, Glt</td>
<td>Leu</td>
<td>Cys, Cys</td>
<td></td>
<td></td>
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<tr>
<td>Theoretical mass</td>
<td></td>
<td>(57.1, 147.1) (99.3, 113.7) (114.1, 99.3, 128.1, 128.1) (137.2, 113.7)</td>
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<td>265.3</td>
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</tbody>
</table>

The coupling reaction was carried out in a 1:1 mixture of 0.1 M ammonium carbonate and pyridine containing 5% PITC (pH 9.0). The reaction mixture was kept at 25°C for 40 minutes. Following cleavage in 100% TFA the reaction mixture was lyophilized, redissolved in isopropanol:0.1% TFA (1:1) and an aliquot finally applied to the nitrocellulose surface. The first seven degradation-steps was performed with an initial sample amount of app. 500 picomoles.

a) PITC-coupling to the free amino-group at Lys was only partial, but after three coupling-steps the coupled species is by far the most abundant.

Furthermore, the use of alternative isothiocyanate-derivatives in order to obtain higher coupling efficiency/selectivity and/or higher molecular ion yield of the coupling-product, has been evaluated. 4-(4-(dimethylamino)phenylazo) phenylisothiocyanate (DABITC), Benzophenonisothiocyanate (BITC), Malachitegreenisothiocyanate (MITC) and Erythrosinisothiocyanate (EITC) were tested and coupling was found to be quantitative for BITC and EITC, but only 50% for DABITC and MITC at standard coupling conditions. The molecular ion signal intensities generally decreased with the higher molecular weight coupling reagents. PITC thus appears most advantageous for sequencing application, since at the conditions outlined in Table 1 it reacts completely with free amino- and sulfhydryl groups. The coupling selectivity of higher molecular weight isothiocyanate reagents in proteins with intact tertiary structure is investigated at present.

Acknowledgement: Part of this work has been supported by the Humboldt Foundation (Bonn, Germany).

References:
4-Nitrophenylisothiocyanate In Peptide Sequencing By Negative Ion Chemical Ionization Mass Spectrometry
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The suitability of 4-nitrophenyllisothiocyanate (NPITC) as a modified Edman reagent in peptide sequencing by Negative Ion Chemical Ionization Mass Spectrometry (NICI MS) has been proposed elsewhere (1).NICI mass spectra of most of the nitrophenylthiohydantoin (NPTH) derivatives gave only the molecular anions with no fragmentation. Hence, the amino acid residue can be detected by the diagnostic mass.

Initial rates of the coupling reaction between the two isothiocyanates (PITC and NPITC) with alanine were compared. NPITC coupled about 6X faster than PITC. It has been reported that NPTC-amino acids are about 3X slower to cleave than the PTC-analogues (2). Therefore, the advantage of having faster coupling rates with NPITC is partially offset by the slower cleavage rates.

Coupling yields of NPITC with amino acids reached between 92-98% (Fig.1) with NPITC to amino acid ratio of 2:1. Under similar conditions, PITC gave a yield between 78-80% (Fig.1). In a typical sequencing experiment 100-1000 molar excess of isothiocyanate is usually used. Therefore, with NPITC, a potential exists for faster sequencing cycles.

Detection limits of NPTH-amino acids were studied in both full scan (250 femtomoles) and single ion monitoring (50 femtomoles). S/N ratios observed were high enough to go to lower levels of detection. Detection limits calculated according to the method given in HP 5985 B GC-MS manual, less than 10 femtomoles can be detected with single ion monitoring.

Manual sequencing of 50 nanomoles of bradykinin with NPITC to peptide ratio of 8:1 gave a repetitive yield of 91% (Fig.2). Total cycle time was 60 min. Lower levels of peptides may be sequenced by using a higher ratio of NPITC to peptide and by optimizing the reaction conditions. This may also allow to reduce the total cycle time. Automated sequencing of 2 nanomoles of β-lactoglobulin gave a repetitive yield of 92% (Fig.3). Total cycle time and reaction conditions used were very much similar to that used with PITC, with a few minor changes. After each delivery of NPITC, the lines were washed out with ethylacetate. Post coupling solvent extraction was also done with ethylacetate. Reaction conditions remain to be optimized in order to achieve higher repetitive yields, which in turn will allow sequencing lower levels of peptides.

Our results, although preliminary, may indicate the potential for faster sequencing cycles with NPITC. In addition, given that the reaction conditions can be optimized to achieve higher repetitive yields, the low detection limits observed with NPTH-amino acids may allow sequencing of low levels of peptides.

References

Acknowledgements: This work was supported by NSF (DIR 8704236). Will Burkhart's assistance with the sequencer and various assistance provided by Larry Shampine and Sue Brackett is gratefully acknowledged.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Figure 1. Coupling yields of PITC and NPITC with Methionine

Figure 2. Repetitive yield of bradykinin in manual sequencing

Figure 3. Repetitive yield of β-lactoglobulin in automated sequencing
A NOVEL APPROACH FOR SEQUENCING OF PEPTIDES:
PARTIAL HYDRAZINOLYSIS FOLLOWED BY MASS SPECTROMETRY

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Introduction
The most commonly used technique for peptide sequencing is automated Edman degradation. The long analysis time, difficulty to analyse mixtures and detect modified amino acids are major drawbacks of this method. Mass spectrometry has found an increasing use in mass mapping and sequencing of peptides over the last few years. The sequence analysis by tandem mass spectrometry may be very rapid, but success and sensitivity are variable and limited by the size of the peptide. Here we present preliminary studies of a different approach that could supplement the above methods.

Method
The peptide (here: glucagon or bradykinin) was first partially degraded by treatment with anhydrous hydrazine at 100 °C [1]. The reaction time was varied between 5 minutes and 8 hours. The C-terminal fragments of the generated mixture are free peptides whereas all other fragments contain hydrazides:

\[
\text{R-CO-NH-R'} \rightarrow \text{R-CO-NH-NH}_2 + \text{NH}_2-\text{R'}
\]

After removal of the hydrazides by binding them to an immobilized aldehyde (made by oxydizing Sepharose with periodate [2]), a "ladder" of C-terminal peptides was left which differ by the number of amino acids on their N-terminal side. Finally the mass of each component in the resulting peptide mixture was determined. This was done either directly or after partial HPLC separation. Matrix assisted laser desorption time-of-flight mass spectrometry with sinapinic acid as matrix [3] was used for analysis of the complex mixtures generated from glucagon. HPLC separated fractions were analysed by liquid secondary ion mass spectrometry on a Kratos MS50 instrument.

Results and Discussion
The time required for the ideal degree of fragmentation (cleavage at one peptide bond per molecule) was expected to be much shorter than required for complete hydrazinolysis. Accordingly, 15 minute treatment of glucagon generated an array of C-terminal fragments that covered about 40% of the sequence (Fig. 1). A side reaction converting arginine to ornithine occured rapidly but did not prevent the analysis. When glucagon was treated for 30 minutes with hydrazine an increased cleavage of the side chains of asparagine and glutamine was observed. Since this side reaction converted most molecules to hydrazides they were removed by the immobilized aldehyde. For bradykinin again arginine was rapidly converted to ornithine. The best conditions were found to be a 90 minutes hydrazinolysis which gave about 50% of the whole sequence.
The simplicity of the method and the small amount of sample used in the final mass mapping step suggest that this approach may be useful for partial sequencing of peptides. The method could also be used for larger peptides or intact proteins without prior enzymatic digestion. Here the limitation is the mass determination. At present matrix assisted laser desorption mass spectrometry is most useful due to its capability to analyse mixtures, but the mass accuracy is limited. We expect that the development of improved techniques for high mass analysis will increase the utility of this method.

References

Acknowledgements
This work was supported by NIH Division of Research Resources Grant #RR01614 (A.L. Burlingame, P.I.) and the Cigarette and Tobacco Surtax Fund of the State of California through TRDRP of University of California (H. Leffler, P.I.).

Fig. 1. Glucagon (8 nmol) was treated with anhydrous hydrazine for 15 minutes at 100 °C. The hydrazides were removed using an immobilized aldehyde (oxydized Sepharose). The remaining mixture of C-terminal peptides was partially purified by HPLC and an aliquot of 0.1 pmol was analysed by matrix assisted laser desorption mass spectrometry. The inset shows the sequence of glucagon (G) and some hydrazinolysis products (G', G'-n). Note that arginine (in G) was rapidly converted to ornithine (in G').
Dimethylaminomethylene Derivatives of Peptides for Mass Spectrometric Analysis.

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Derivatization is often employed in mass spectrometry (MS) in order to increase volatility, ionization yield, or specificity (1). Several derivatives have been demonstrated to be useful also in peptide analysis (2). We found that the dimethylaminomethyl derivatives of peptides have interesting characteristics that may analytically be exploited.

The derivatizing agent is dimethylformamide dimethylacetal, an often used reagent for carboxylic acids and amino acids in gas chromatographic analyses. It reacts smoothly with primary amino moieties turning them into dimethylaminomethylene groups. We derivatized several commercially available peptides with amino acid residues between 3 and 10 (such as trialanine, leu-enkephalin, angiotensin-ii, bradykinin), and found the following:

1. The derivatization can be carried out in micro-scale, e.g., on the fast atom bombardment (FAB) probe tip, with the peptide samples dissolved in glycerol. Water-free conditions are not required.
2. The derivative-formation is largely completed within one minute. Each primary amino-group (the terminal one and those in the basic amino acids' side chain) contributes 55 amu to the increase of the molecular weight.
3. The intensity of the derivatized spectra (TIC) is in general higher than those of the un-derivatized ones, and the molecular ion is the base ion.
4. The solubility of the analyte increases during derivatization, thus analysis can successfully be performed with peptides suspended in the glycerol matrix (e.g., p-nitrophenyl glutathione).
5. The low-energy daughter ion spectra are somewhat more complex than those obtained from the un-derivatized peptides, but still may be useful for sequence studies.

In conclusion, the dimethylaminomethylene peptide derivatives are interesting compounds that may serve well for molecular weight confirmation, for counting primary amino groups, and perhaps for sequencing, too. However, the conditions of reproducible derivative-formation should be explored.

References

2. D.F. Hunt et al., ibid., Chapter 5, p.186.
The introduction of the continuous-flow probe has greatly facilitated the analysis of synthetic peptides and peptides derived from chemical or enzymatic fragmentation of proteins. Most applications of the flowprobe in protein analysis center around the determination of masses of peptides obtained with FAB or SIMS techniques. Peptide sequence analysis generally requires tandem MS techniques. CAD/Linked-scan procedures with two-sector instruments using 1FFR collision activation can also provide sequence ions. Such analyses are usually performed using peptides dissolved in a liquid matrix.

We have obtained sequence information from synthetic peptides by CAD/linked-scan analysis using a flowprobe with direct injection of the sample into the source. The peptides are analyzed in two steps. A first injection with conventional magnetic scanning establishes the masses of candidate parent ions. After a second injection, the peptides are analyzed by linked-scan analysis of daughters generated by 1FFR collision with helium. Peptides were analyzed with a Finnegan MAT 90, equipped with a continuous-flow interface which was modified to accept a stainless steel frit at the target. A syringe pump HPLC delivered solvent (0.1% TFA, 2% glycerol, 3% acetonitrile in Water) at 10 μl/min to the probe. Injections of 0.5 μl containing several micrograms of peptide dissolved in LC solvent were performed every 2 minutes. Scans of constant B/E were acquired at 15 sec/decade. The source temperature was 75°. A "chromatogram" plot of the injections of several peptides is shown in Fig. 1. Accumulated spectra of three peptides is shown in Figs. 2-4, along with their predicted ion series.

The utility of LC/CAD analysis was demonstrated using a peptide with a synthetic defect. The nonamer Cys-Gly-Ser-His-Leu-Val-Glu-Ala-Leu was prepared by solid-phase peptide synthesis using the NMP/HOBt method. This synthetic procedure is widely used and has been shown to produce peptides with higher average coupling efficiencies than other methods. A Bom-protected Boc-Histidine was used in the initial synthetic trials. FABMS analysis indicated the presence of the normal peptide at m/z 928, plus a significant ion at m/z 940, 12 amu higher than predicted. In the spectra obtained from some peptide preparations, the higher mass ion predominated. Sequence ions from m/z 928, obtained by linked scans using LC/CAD analysis showed the predicted b series expected for the normal peptide (not shown). Some spectra also showed a weak, overlapping y series which, together with the b series, provided complete sequence information for the peptide. A preparation of the modified peptide was then analyzed for daughters of m/z 940 using LC/CAD. The spectrum from accumulated scans of one of the injections is shown in Fig. 5. In this spectrum, the daughters of m/z 940 were consistent with a b series, starting with histidine, that is 12 amu higher than that predicted for the normal peptide. No other ion series was observed.

When the peptide was resynthesized using Dnp-protected Boc histidine, the mass spectra of the product showed no traces of the +12 amu ion. The Dnp protecting group must be removed prior to cleavage by HF. One possible interpretation of the results is that during the cleavage of the Bom-protected peptide form the PAM resin by HF, an imidazole nitrogen of histidine becomes methylated (Fig. 7). This might lead to the formation of an unprotonated molecular ion (M+) because of charge stabilization on the imidazole ring. It would also predict a peptide molecular ion and b4 - b5 series exactly 12 amu higher than expected for the normal peptide. An alternative explanation for this +12 amu modification is the conversion of the N-terminal cysteine to thioproline by formaldehyde. 4 Formaldehyde is liberated from Bom-Histidine during the cleavage step. While we cannot rule out this modification in the case of peptide 225, we have observed the +12 amu modification in other His-containing peptides which do not contain cysteine.


Acknowledgements
We wish to thank William Bear for producing the synthetic peptides, Eric Wexler for the program "Sequon" and B. Pramanik (Schering Corp.) for his helpful suggestions.

Abbreviations: Bom- Benzyloxymethyl, Boc- t-butyloxycarbonyl, Dnp- 2,4-Dinitrophenyl, NMP- N-Methylpyrrolidone
HOBt- 1-Hydroxybenzotriazole, DCC- Dicyclohexylcarbodiimide
Figure 1. CAD analysis of synthetic peptides

Figure 2. Peptide #85 TTLVLSGE

Figure 3. Peptide #158 DLRGYVYOGL

Figure 4. Peptide #211 CIKVAVS

Figure 5. Modified Peptide 225 CGSHLVEAL

Figure 6. Predicted Peptide Sequence Ions

Figure 7. Possible Histidine structures in Fig. 5. A: Normal His in protonated peptide. B & C. Methylated imidazole nitrogen would yield an unprotonated molecular ion 12 amu higher than for A.
DETERMINATION OF RELATIVE RATES OF HYDROLYSIS OF BIOACTIVE PEPTIDES BY DIGESTIVE PROTEASES USING CONTINUOUS-FLOW FAST ATOM BOMBARDMENT MASS SPECTROMETRY.

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Many drugs under development contain peptide bonds or are peptide analogs. For oral administration(1), these drugs should resist hydrolysis by the digestive system. We have developed an in vitro assay using immobilized digestive enzymes and CF-FAB/MS with selected ion monitoring (SIM) to rapidly determine whether these compounds will be hydrolyzed and, if so, how rapidly they will be degraded. Previously(2), identification of hydrolysis products of immobilized enzyme incubations was demonstrated using frit-FAB LC/MS. CF-FAB/MS (with and without an internal standard) and HPLC with UV absorbance detection will be compared for the determination of hydrolysis rates of bioactive peptides by immobilized digestive proteases.

Porous glass beads were silanized, succinylated, and activated by 3-(3-dimethylaminopropyl)carbodiimide. Trypsin and chymotrypsin were separately immobilized(3). Enzyme activities were determined spectrophotometrically by monitoring the release of chromophore-containing products from peptide analogs(4). The in vitro assay using immobilized trypsin and chymotrypsin will be used to simulate digestion of three bioactive peptides in the 8 intestinal lumen. Angiotensin II, \([\text{Arg}^2]-\text{vasopressin}\), and \([\text{deamino-Cys}^8, \text{D-Arg}^9]-\text{vasopressin}\) will be individually incubated with each immobilized enzyme in 0.1 M ammonium acetate pH 7.5 at 37°C. Substrate-to-enzyme ratios of 50 to 1 and 200 to 1 were used for trypsin and chymotrypsin respectively. Ten microliter aliquots were withdrawn at regular intervals. Separate digestions were run for HPLC and CF-FAB/MS analysis.

Aliquots from each incubation were analyzed by reversed phase HPLC. A hybrid HPLC system consisting of Water's Model 501 pumps, Applied Biosystems (Foster City, CA) 757 UV detector, a Rheodyne (Cotati, CA) Model 7125 injector and a Vydac (Hesperia, CA) CIS column (4.1 mm x 25 cm). Various gradients were used to obtain complete resolution of the initial substrate from all hydrolysis products. Solvents were water and acetonitrile each containing 0.1% TFA.

The CF-FAB/MS system consisted of an Applied Biosystem Model 140A dual syringe delivery system equipped with a Rheodyne model 8125 injector and a JEOL HX110HF double focusing mass spectrometer equipped with a DA-5000 data system and frit-FAB interface. Fused silica capillary (0.75 mm id.) connected the injector with the frit-FAB interface. A carrier solution of water:acetonitrile:TFA:glycerol (80:20:0.1:0.5) was used to deliver incubation aliquots onto the ionization stage at 5uL/min. SIM was used for quantification.

The in vitro assay using immobilized enzymes, HPLC and/or CF-FAB/MS with SIM is amenable for hydrolysis rate determinations of bioactive peptides by digestive proteases. Rate determinations by HPLC and CF-FAB/MS with an internal standard yield statistically identical results.(table 1) The correlation coefficients, and error limits for both methods are similar. The separation requirement for HPLC limits its ability for rate determinations for some peptides (figure 1 and 2). For CF-FAB/MS, separation of hydrolys products from the initial substrate is not required and this method can be used to complement HPLC or as an alternative to it (table1 and figure 3). CF-FAB/MS also provides molecular weight information on hydrolysis products. It has been demonstrated that this assay can be used to identify enzymatic hydrolysis products of orally administered peptide drugs and to determine their hydrolysis rates. The assay can be used to assist pharmacologists in drug design.
Figure 1. HPLC analysis of AVP incubated with immobilized trypsin. The labeled peak contains AVP and a hydrolysis product as determined by positive FAB/MS (see figure 5). Gradient variations failed to obtain completely resolution with the Vydac C18 column. Hydrolysis rate determinations by HPLC are difficult in these cases where the substrate is not completely resolved.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Chymotrypsin</th>
<th>Trypsin</th>
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<tr>
<td>Angiotensin II</td>
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<td>140 (+/-5)</td>
</tr>
<tr>
<td>DDAVP</td>
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<td>6840 (+/-1080)</td>
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<tr>
<td>AVP</td>
<td>9 (+/-1)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Detected by CP-FAB/MS

REFERENCES

Analysis of Hydrophobic Peptides by Liquid Secondary Ion Mass Spectrometry

Patrick R. Jones, Arnold M. Falick and Larry J. W. Miercke

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The application of mass spectrometry to the mapping and sequencing of proteins and peptides is well established. Liquid secondary ion mass spectrometry (LSIMS) methods generally work well for hydrophilic and moderately hydrophobic peptides, although hydrophilic peptides may require derivatization to make the compound more abundant on the surface of the liquid matrix material. Problems arise with more hydrophobic peptides which tend to aggregate at higher concentrations in aqueous solution or be insoluble. A digest of the protein of interest is carried out to provide tractable peptide fragments for LSIMS. The peptides are separated by HPLC and mass analyzed. In a typical water/acetonitrile gradient elution scheme on a reversed phase column with 0.1% trifluoroacetic acid (TFA) added, the peptides found in the initial portion (up to an HPLC Index of about 30 based on a calculation using the MacProMass program supplied by Dr. Terry Lee) may need derivatization. Those in the range 30 - 115 give LSIMS spectra readily, but those beyond 115 often give no appreciable signal.

We chose as a model, for work on extending the applicable range of LSIMS, bacteriorhodopsin, a membrane protein. A combination of CNBr and trypsin digestion schemes were used. The conventional HPLC analysis with C4 and/or C18 columns using a water-acetonitrile-0.1% TFA gradient proved unsatisfactory for the determination of all the peptide fragments. In part, this system is problematic since many of the peptide fragments are significantly hydrophobic. In addition, the peptides tend to precipitate in the HPLC and give troublesome residues that are difficult to elute.

An HPLC method using a water-acetonitrile-5% formic acid gradient proved to work well. The more hydrophobic peptides were collected and found to be amenable to LSIMS analysis on a Kratos MS50S mass spectrometer when the lyophilized samples were taken up in 96% formic acid just prior to analysis. Hydrophobic samples extracted with water-acetonitrile-0.1% TFA gave no peptide ion signals by LSIMS. The peptide sequences were confirmed by high energy collision induced dissociation in a Kratos Concept four sector tandem mass spectrometer.

Supported by NIH grant DRR 01614 and NSF grant DIR 8700766.
References:

Figure 1. HPLC Chromatogram of a CNBr Digest of the C2 Fragment of Bacteriorhodopsin on a C4 Column with a Water-Acetonitrile, 5% Formic Acid, Linear Gradient of 0.5%/min, 280nm.

Figure 2. LSIMS Spectrum of the pGluAQITGRPEWIWLALGTALHse Peptide, of Bacteriorhodopsin (CNBr-2, MH⁺ = 2190.2, HPLC Index 128)

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The recent development of the matrix-assisted UV laser desorption technique has enabled the formation and subsequent mass analysis of very large (>100 kDa) protein ions [1]. Most instrument designs have implemented costly Nd:YAG lasers typically equipped with frequency tripling and/or quadrupling devices to produce UV radiation. A linear TOF instrument that utilizes a multi-gas laser with a N2 lasing mixture as the UV photon source (337 nm) is shown in Figure 1. Using matrices such as sinapic acid, which exhibits strong solid-phase optical absorption at 337 nm, we have successfully desorbed and ionized proteins having high molecular weights with sensitivity in the low picomole range. Nitrogen lasers capable of producing the required irradiance levels (~1 MW/cm²) may be purchased for ~$10,000. A nitrogen laser system offers the advantages of a less complex optical system (the fundamental frequency is used), shorter pulse widths, higher repetition rates, and substantially lower cost when compared to a Nd:YAG laser.

**Charge State/Kinetic Energy Effects**

Figure 2 shows a portion of the mass spectrum of horse heart cytochrome c (MW 12,360) in sinapic acid analyzed with a nominal ion source bias of +30 kV. The apparent resolution (R= t/2At FWHM) of the M⁺¹ quasimolecular ion region is 140. However, the resolution of the M⁺² region is 280. This may be explained by considering the initial kinetic energy of the ions formed by the desorption/ionization process. The nominal kinetic energy (E) of the singly charged ions is 30 keV, while the nominal kinetic energy of the doubly charged species is 60 keV. If the greatest contribution to peak broadening is the initial kinetic energy spread (ΔE), the resolution may expressed as [2]

\[
R = \frac{t}{2\Delta t} = \frac{E}{\Delta E} \quad \text{(mass independent)}
\]

If the limiting factor in mass spectral resolution arises from initial kinetic energy, then these data suggest that the initial kinetic energy spread is in excess of 100 eV.

**Matrix/Analyte Molar Ratio Effects**

A series of solutions of varying matrix/analyte molar ratio were prepared using horse heart cytochrome c as the analyte protein and sinapic acid as the matrix. Samples were prepared by applying a 1µl drop of the matrix/analyte solution to the probe tip and drying under a N₂ stream. The protein loading and the laser irradiance were held constant for each analysis. The probe tip was rotated while a 500 shot averaged spectrum was acquired to provide uniform sampling of the surface. Matrix associated (low mass) and analyte M⁺¹ and M⁺² peaks were integrated. The absolute intensity of the matrix and analyte ion signals and the ratio of the analyte/matrix signals are plotted as a function of the matrix/analyte molar ratio in Figure 3. With the exception of the lowest (100:1) and the highest (10,000:1) relative concentrations, both the absolute and the relative abundances of the matrix and analyte ion signals remain relatively constant.

Utility of a cysteine-specific cleavage reaction in the structure determination of proteins by laser desorption time-of-flight and tandem mass spectrometry

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Introduction. It has been known for some time that polypeptides can be cleaved at the N-terminus of cysteine after cyanation. The formation of an iminothiazolidine ring at the product N-terminus prevents sequencing by conventional Edman techniques. Further development of the cyanation and cleavage was thus delayed until introduction of tandem mass spectrometric sequencing. Peptides in the range of m/z 3000 or less can be sequenced with present technology, and it has been found that cysteine cleavage products in this range tend to yield complete sequence data, dominated by a and b ions.

Larger cleavage fragments can now be analyzed by employing the new matrix-assisted laser desorption (LD-TOF) technology. The reaction can be monitored for completeness with little or no cleanup required for a mass determination. The completed reaction can be desalted to yield sharper, adduct free peaks.

Method. The cyanation can be carried out in two different ways:

1. Electrophilic cyanate.
   Here the cysteine is reduced before reaction with the electrophilic cyanate reagent. The sulfhydryl acts as nucleophile to attack the cyanate moiety. 2-Nitro-S-thiocyanatobenzoic acid (NTCB) has been used in the past, but we have found 4-thiocyanatoaniline (TCNA) to be a useful alternative. The polypeptide is reduced, and cyanlated at pH 8, and then dialyzed or extracted to remove the excess of the peptide bond N-terminal to the original cysteine occurs at pH 9 at 37 °C.

2. Nucleophilic cyanide
   Cyanide ion is used to reduce the disulfide bond directly. One cysteine is cyanylated, and the other is left as a free thiol. The cleanup of the reaction is minimal, requiring only a desalting HPLC step prior to tandem mass spectrometry, and nothing at all for LD-TOF. The easy cleanup is a distinct advantage for very small peptides for which dialysis is impossible and extraction risky. For larger polypeptides with disulfide bonds, it may be possible to use this method of cyanylation and cleavage to determine location of the disulfide bonds.

Tandem Mass Spectrometry was performed on a JEL HX110/110 double focusing tandem mass spectrometer, which is of E^E,!^ configuration. Collision induced dissociation of the protonated peptide molecule selected with MS-1 took place in the field-free region after Bp. The collision cell potential was 3.0 kV and the ion collision energies were at 7.0 keV. Helium, the collision gas, was introduced into the collision chamber so as to reduce the precursor ion signal by 65-70%. 100 Hz filtering was used with a scan rate of m/z 0 to 6000 in 15 min. Only the C12 species of the protonated peptide molecule was transmitted through MS-1. MS-2 was operated at 1000 resolution and was calibrated with a mixture of CsI, NaI, KI, RbI, and LiCl.

Matrix-Assisted Laser Desorption Time of Flight Mass Spectrometry was performed on a Vestec VT2000 instrument. The 355nm, frequency tripled output of a Nd^YAG laser was used to ionize the sample. The laser output pulse length was approximately 15 ns, the irradiance used was approximately 10MW/cm^2, and the laser was operated at 5 Hz. The matrix used was sinapinic acid, and 1-2 pmol of analyte were placed on the probe tip. The instrument was operated at 30KV accelerating voltage. Spectra were obtained by adding together 40 laser shots using a transient recorder, which was controlled by a PC. The data were stored temporarily on the PC and then transferred to a VAX work station for analysis. Software for calibration, analysis, and presentation of the data were developed in our laboratory (see MP 111).

Results. Figure 1 shows the LD-TOF mass spectrum of human hemoglobin α chain which has been cleaved with the TCNA reagent. The average protonated molecular weights for the theoretical products and those found by the use of external calibration are,

<table>
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<tr>
<td>1-103</td>
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<td>1-141</td>
<td>15127.38</td>
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</table>

and are easily identified from the spectrum.

The N-terminal Figure 2 shows the C-terminal peptide resulting from the cyanation and cleavage of ribonuclease A. The spectrum exhibits an a ion at m/z=101 which is indicative of the presence of iminothiazolidine at the N-terminus and is dominated by a and b ions which reveal the complete sequence.

Isotocin (CYSNCPIC) was used to demonstrate the cyanide ion induced reduction and cyanylation on a small peptide. Isotocin is typical of neuropeptides, having an N-terminal cysteine with a disulfide bridge. Cyanide ion alkylating at the cys-1 half of the disulfide bond
yields an iminothiazolidine ring at the N-terminus but does not involve a peptide bond cleavage. The CID spectrum (not shown) is dominated by a and b ions and contains complete sequence information. The use of cyanide salts for these small peptides is convenient due to the minimal cleanup required.

The treatment of larger polypeptides with cyanide salts may allow the assignment of the disulfide bonds. For a protein with two disulfides, there exist three possible disulfide bonding distributions. If a peptide is detected that is not possible for a given distribution, that distribution is eliminated from consideration.

Conclusion. Polypeptides can be cleaved at cysteine with the NTCB or TCNA reagents. For large products mass assignments can be made with the LD-TOF instrument, allowing for monitoring of the reaction as it progresses. Smaller peptide products can be sequenced with the tandem mass spectrometer.

Cyanide ion is preferable for smaller cysteine containing peptides where removal of the excess cyanate reagents proves difficult. The presence of N-terminal iminothiazolidine appears to direct fragmentation towards the production of a and b ions.

The reaction with cyanide ion may allow the assignment of the distribution of disulfide bonds in proteins.


Figure 1.

Figure 2.
Analysis of Highly Glycosylated or Hydrophobic Membrane Proteins by Laser Desorption Time-Of-Flight Mass Spectrometry

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Introduction.

Purification and structural analysis of highly glycosylated proteins or of hydrophobic membrane proteins present unique challenges to the protein chemist. Presence of carbohydrate complicates chromatography, proteolytic digestions, and structural analysis of the resulting glycopeptides. Glycoproteins often give broad, poorly defined bands by SDS-gel electrophoresis or gel permeation chromatography producing unreliable estimations of molecular weights. For the hydrophobic proteins, solubility is the problem and the use of detergents for purification and further analysis is generally required. As a consequence of these features, the utility of one of the most important contemporary analytical tools to the protein chemist, namely mass spectrometry, is compromised. As part of a continuing effort in the protein structural field, we have recently begun to use laser desorption time-of-flight mass spectrometry to analyze these types of proteins. We report here on some of the uses and limitations of this approach.

Materials and Methods.

Ribonuclease-B, ovalbumin, fetuin, and lactoferrin were purchased from Sigma. The NCA-50, NCA-75, and carcinoembryonic antigen (CEA) samples were isolated from a liver metastasis of a colonic tumor. For analysis by LD-TOF MS, these proteins were dissolved in 0.1% TFA and mixed with an equal volume of a saturated solution of 3,5-dimethoxy-4-hydroxy-cinnamic acid (sinapinic acid) dissolved in 30% aq. CH3CN, 0.1% TFA. An aliquot (generally ~1 ul) of the sample/matrix was spotted on a copper stage and allowed to air dry. Mass analysis was performed using a Shimadzu LAM-50KS LD-TOF mass spectrometer equipped with a Nd:YAG and operated at 355 nm.

Nine different cytochrome P450 samples were isolated from natural sources. Successful analysis in most instances required the removal of interfering components. Sample solutions were dried on the bare copper sample stage in vacuum and then washed with a solution of aqueous 30% acetonitrile, 0.1% TFA. Sinapinic acid matrix was added to the sample and mass spectra were collected as described above.

Results

LD-TOF MS of glycoproteins. The general trend observed was that as the complexity and amount of carbohydrate present increased, the molecular ion signal broadened reflecting a multiplicity of ions arising from microheterogeneity of glycosylation and matrix adduct formation. For example, for Ribonuclease-B (m/z 13572, one glycosylation site) the base of the molecular ion signal was 3000 amu wide, but for CEA (m/z 127,222, 28 glycosylation sites) the base width was >20,000 amu. Deglycosylation of the samples significantly increased the signal intensity and sharpness of the molecular ion peak.

Of particular interest in these analyses were the results for the LD-TOF MS of NCA-50 (m/z 46052) and NCA-75 (m/z 65594). These glycoproteins are expressed in granulocytes, the immune phagocytic cells, and function as bacterial binding receptors. They are identical.
with respect to protein sequence and numbers of glycosylation sites (12 N-linked sites) but differ in the types of carbohydrates present. These differences in carbohydrates are functionally important and directly affect binding specificities. In the case of NCA-50, the high mannose-types of carbohydrates are functionally relevant. To examine the amount of such structures, we treated NCA-50 with Endoglycosidase-H to remove high mannose-type structures and with trifluoromethanesulfonic acid to totally deglycosylate the sample. Endo-H treatment shifted the mass by 4444 amu while total deglycosylation caused a 11570 amu mass shift. These results indicate that NCA-50 contains approximately 40% high mannose-type of oligosaccharides.

**LD-TOF MS of Cytochrome P450 Enzymes.** Beavis and Chait have described a simple washing technique in conjunction with the use of sinipinic acid as a matrix to prepare samples for LDMS analysis. This technique proved ineffective for a large majority of the cytochrome P450 samples we have attempted to analyze. The alternative sample washing method described above was more effective for this class of compounds. Cytochrome P450D failed to yield spectra by any of the methods of washing the sample on the metal probe tip. In an effort to further immobilize the sample in order to wash with less polar solvents, the sample solution was spotted on a thin piece of polyvinylidenedifluoride (PVDF) membrane. After vacuum drying, the membrane was washed with 30% aqueous acetonitrile containing 0.1% trifluoroacetic acid. The sinipinic acid matrix solution was added to the membrane and allowed to air dry. Using this procedure, a reasonably good spectrum was obtained. No spectrum was obtained from PVDF without washing, and a very weak spectrum was obtained if the membrane was washed with cold water after applying the matrix. The use of the PVDF membrane introduces an additional complication. Because the membrane forms an insulating layer on the sample stage, the acceleration experienced by the desorbed ions is less resulting in longer flight times and artificially high mass values. If this technique proves generally useful, it will be necessary to have a separate calibration for samples on PVDF or include an internal standard.

**Conclusions**

Heavily glycosylated proteins invariably exist as a multiplicity of species that differ in the number of sugar units attached. Thus far, we have had no success analyzing members of the CEA gene family by electrospray mass spectrometry. We have had some success using laser desorption. The resolution of our instrument is not sufficient to separate the glycoforms. However, even the broad signals observed yield more accurate mass values than can be obtained by SDS page gel electrophoresis and the amount of sample required is generally less. The action of various carbohydrate cleaving enzymes can be followed by observing the mass shifts. As carbohydrate is removed, molecular ion signals narrow significantly and mass accuracy increases.

Hydrophobic membrane proteins pose an entirely different set of problems. Samples are generally heavily contaminated with interfering substances that must be removed for a successful mass measurement. In a few instances, electrospray data can be obtained using on-line LCMS analysis. For these measurements, the mass accuracy is very good. Using novel washing techniques involving binding of the protein to metal and fluorocarbon surfaces, it is possible to obtain laser desorption spectra of Cytochrome P450 enzymes accurate to within 1% or less. Laser desorption mass spectrometry is a nearly universal technique for the analysis of proteins. However, proper sample preparation is of great importance and in many ways mysterious.
INTRODUCTION

Peptide mapping by reversed-phase microbore liquid chromatography combined with ion spray mass spectrometry was used to determine carbohydrate composition and glycosylation sites of glycoproteins. Glycopeptides in proteolytic digest mixtures were identified by LC/MS/MS precursor ion scans. Utilization of collision-induced dissociation (CID) in the free-jet expansion declustering region of the atmospheric pressure ionization source enabled screening for glycopeptides in digest mixtures from full-scan LC/CID/MS experiments which contained the diagnostic ions (m/z 204, 366) and intact precursor ion information in the same data run.

The combination of CID before mass filtering and MS/MS was utilized for the identification of N-glycosylated peptides by: 1) exoglycosidase treatment of glycopeptides to reduce the carbohydrates to a common chitobiose core containing mannose residues, 2) CID in the declustering region to produce a singly charged peptide with one pendent GlcNAc, and 3) MS/MS to remove the GlcNAc as a neutral (-203) loss.

EXPERIMENTAL

A Sciex API III (retrofitted from TAGA 6000E) triple quadrupole mass spectrometer and an Applied Biosystems 140A micropump equipped with a YMC MAQ-110 1 x 100 mm C18 column was used with gradients from 0-50% CH3CN/H2O containing 0.05% TFA at flows of 40 µL/min. Typically, 0.5–10 nmol of glycopeptide digest mixture was injected in 5–20 µL of mobile phase and the gradient increased at 1% CH3CN/min.

Glycoproteins, proteases, and exoglycosidases were purchased from Sigma, and Staph. aureus protease V8 was purchased from Boehringer Mannheim. Ribonuclease (RNase) B was persulfonated by the method of Thannhauser; asialofetuin (ASF) and ovomucoid were reduced and carboxymethylated (RCM). Each was digested with trypsin; RCM ovomucoid and RCM ASF were also digested with chymotrypsin, and the latter was digested with Staph. aureus protease V8 (enzyme:substrate 1:50). Proteolytic digest mixtures were subsequently treated with α-mannosidase and β-N-acetylglucosaminidase in NH4HCO3, Na2HPO4, or NaOAc buffers (50-200 mM).

Data were acquired (6 sec/scan) at m/z 200–2000 for LC/CID/MS (with unit resolution at 50% valley), m/z 400–2400 for LC/MS/MS precursor ion scans and m/z 600–1600 for neutral loss scans (2–3 amu FWHM). Declustering energy in the orifice-to-RF-only quadrupole region (Q0) was 120–140 eV and collision energy in the RF-only quadrupole (Q2) between the tandem mass-resolving quadrupoles was 80–100 eV referenced to the laboratory frame for a doubly charged ion. Argon was used as a collision gas in Q2 at 200-230 x 1012 atoms/cm2, and ultra-high purity nitrogen was used as a declustering gas in Q0. MS/MS product ion spectra were obtained by continuous infusion in MCA mode (64 scans) of peptides which had been fractionated by solid-phase extraction or by LC/MS/MS of the digest mixture.

RESULTS AND DISCUSSION

LC/MS/MS identified glycopeptides containing HexNAc or HexHexNAc units in digest mixtures by precursor ion scans of the characteristic oxonium ions at m/z 204 and 366, respectively. The selectivity of MS/MS enabled the detection of a minor glycoform of ovomucoid containing a terminal galactose on a N-acetylglucosamine2 by a m/z 366 precursor scan. However, resolution was sacrificed in order to achieve adequate sensitivity.

Utilization of collision-induced dissociation (CID) in the declustering region (Q0) of the atmospheric pressure ionization mass spectrometer provided both oxonium ions and intact glycopeptide ions before mass resolution in Q1. LC/CID/MS with declustering energies of 120–140 eV caused both fragmentation of glycopeptides to oxonium ions and improved transmission of intact doubly charged glycopeptides (m/z 750–1500). A single LC/MS run yielded both diagnostic ions (m/z 204, 366) to screen for glycopeptides in mixtures and the distributions of the glycoforms with high sensitivity and unit resolution.
MS/MS of doubly charged tryptic-like glycopeptides containing N-linked carbohydrate yielded oxonium ions along with singly charged reducing-end fragment ions resulting from cleavage at glycosidic bonds. Intact peptide with N-linked GlcNAc was typically a major ion; little or no peptide bond cleavage was seen due to the relative strengths of amide vs. O-glycosidic bonds. Continuous infusion with signal averaging of peptides fractionated with solid-phase extraction produced better quality mass spectra; however, LC/MS/MS could be utilized if sample was not limited.

LC/CID/MS/MS was utilized to selectively determine N-linked glycosylated tryptic-like peptides which had carbohydrate reduced by exoglycosidases to a chitobiose core with mannose residues. The remaining O-glycosidic sugars were removed by CID in Q0 to produce singly charged peptides with pendent N-linked GlcNAc; MS/MS in Q2 efficiently cleaved the GlcNAc as a neutral loss (-203). LC/CID/MS/MS was used to determine the MW of the N-linked glycosylated peptides of persulfonated RNase B digested with trypsin and α-mannosidase (Figure 1) and RCM ovomucoid digested with trypsin and β-N-acetylglucosaminidase.

REFERENCES

LC/CID/MS/MS of Asn-34 Glycopeptide from Ribonuclease B
Digested with trypsin and α-mannosidase
Declustering Energy = 120 eV
Collision Energy = 50 eV (for [M + H]⁺)
600,1200 → (-203)

![Diagram of LC/CID/MS/MS of Asn-34 Glycopeptide from Ribonuclease B](image_url)

Figure 1
INTRODUCTION

In plasma desorption mass spectrometry (PDMS) [1], the sample solution is generally applied onto a nitrocellulose-coated, foil target and dried prior to analysis. During mass analysis, only a small fraction of the sample is consumed due to the low flux of primary ions and the high sensitivity of ion detection characteristic of this technique. The very low sample consumption makes it feasible to reuse the sample after mass determination. For example, mass analyses can be performed on the same sample target before and after in-situ enzymatic digestion or chemical reaction. Another approach is to recover the remaining sample from the nitrocellulose matrix for further study. Roepstorff has demonstrated that a peptide sample can be eluted from the nitrocellulose to a PVDF membrane for subsequent microsequencing [2]. We have devised a simple protocol for recovering sample in solution from the nitrocellulose matrix for further structural analyses. The effects of various eluting solutions on sample recovery yield and background contamination are discussed. Results from amino acid analysis, microsequencing and enzymatic digestion of samples recovered from targets after PDMS analysis demonstrate a practical approach to obtaining maximum structural information from a limited amount of peptide or protein sample.

EXPERIMENTAL

Instrumentation Mass analyses were performed using a Biolon™ 20 Biopolymer Mass Analyzer. Automated Edman degradation were performed using a Model 477A Pulsed Liquid Phase Sequencer with on-line model 120A PTH Amino Acid Analyzer. Amino acid compositions were determined using a Model 420H Amino acid Analyzer. (Applied Biosystems, Inc.)

Deglycosylation of peptide. Glycopeptide samples (approximately 200 pmol) were dissolved in 19 μL buffer containing 0.2M Na2PO4 (pH 8.5). One micro-liter (0.25 unit) of N-glycanase (from Genzyme) was added to the solution. The reaction mixture was incubated at 37°C overnight.

EVALUATION OF RECOVERY PROTOCOL

Choice of Method

A simple protocol is shown in Fig. 1 for recovering of samples from a nitrocellulose-coated target into proper solvent. The solution can then be directly applied onto a sequencing membrane, or collected in an Eppendorf tube for enzymatic treatment, HPLC, or amino acid analysis.

Choice of Extraction Solvent

The IPA and TFA were chosen because of the low solubility of nitrocellulose in these solvents resulting in less contamination during extraction [3, 2]. The effects of organic solvent concentration and pH of the extraction solution on the recovery yield and contamination were evaluated. For peptides, the results indicate typical recovery of 50 to 95% (not shown). Higher concentrations of isopropyl alcohol improve the recovery of peptides with longer retention time on the reverse-phase HPLC system. For protein samples, the recovery yield is more sample dependent. The solvent effects were evaluated using calmodulin (from porcine brain) as a model compound (Table 1). Other proteins, such as beta-lactoglobulin, lysozyme, insulin, extracted with standard conditions (70%IPA, 5%TFA) resulted in 30 to 90% recovery yield.

Figure 1. Protocol for Recovering Protein/Peptide Samples from Nitrocellulose-Coated Targets

1. Apply 15 to 20 μL Propanol/TFA
2. Equilibrate for 4 min Keep the solvent within the nitrocellulose matrix
3. Recover the solution with care. Avoid damaging the nitrocellulose film
Table 1. Protein sample extraction from nitrocellulose target. Starting material: 134 pmol calmodulin (2.24 μg). The recovered samples were quantitated by amino-acid analysis.

<table>
<thead>
<tr>
<th>Extraction solvent</th>
<th>Recovered Amount</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>%IPA</td>
<td>%TFA</td>
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</tr>
<tr>
<td>50</td>
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<td>50</td>
<td>5</td>
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<tr>
<td>0</td>
<td>5</td>
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</tr>
<tr>
<td>30</td>
<td>5</td>
<td>0.21</td>
</tr>
<tr>
<td>70</td>
<td>5</td>
<td>1.03</td>
</tr>
</tbody>
</table>

INTEGRATION OF PDMS WITH PROTEIN CHEMISTRY
Example: Analysis of Glycopeptide from Tryptic Digest of Carboxypeptidase P
Structural analysis of glycopeptides continues to be a challenge for most protein chemists. Little information can be obtained by conventional sequence analysis except for speculation that the sample may be glycosylated. In this example (Fig. 2), we performed mass analysis of the intact glycopeptide (Fig. 2, top right). The sample was recovered from the target, deglycosylated with N-glycanase, purified (middle left) and analyzed by PDMS (middle right). The mass difference suggested the possible carbohydrate structure. Furthermore, the complete sequence of the peptide was determined by microsequencing (Fig. 2, bottom) the peptide recovered after the second round of mass analysis.

CONCLUSION
- Protein and peptide samples can be recovered from nitrocellulose-coated targets after PDMS. The simple protocol presented here recovers proteins and peptides into IPA/TFA solution for further structural analysis.
- The recovered sample solution can be applied directly onto a pre-conditioned sequencing matrix, or subjected to amino acid analysis without further purification.
- The integration of PDMS with in-situ protease or chemical digestion protocols, microsequencing, amino acid analysis and HPLC provides a powerful tool for solving some of the more complicated protein structure problems.

REFERENCES
Applications of the Parent Scan Mode for the Sequence Analysis of Amino-Terminal Derivatized Peptides

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The determination of a peptide sequence directly from daughter MS/MS spectra involves a systematic process of logical deductions via fragmentation patterns and a knowledge of fragmentation pathways. The process of spectral interpretation is facilitated by the fact that peptides generally undergo characteristic fragmentations. The resulting fragment ions are typically indicative of a particular amino acid. However, this process can often be time-consuming, especially when dealing with an unknown peptide. A limitation of the daughter MS/MS mode for the direct sequence analysis of unknown peptides is the presence of both N-terminal and C-terminal sequence ions in the daughter spectrum. A complementary mass spectrometric method which could provide spectra containing only N-terminal or C-terminal fragment ions would result in rapid sequence determination of unknown amino acid sequences, and perhaps, a simpler and systematic method for amino acid sequence confirmation.

Here, the application of the parent scan mode for the unidirectional sequence analysis of peptides is demonstrated with methionine enkephalin (Figure 1). This method is based on the fact that peptides undergo similar fragmentations corresponding to cleavages through the amide linkages. Specifically, N-terminal and C-terminal substructures undergo characteristic MS/MS fragmentations indicative of the N-terminal amino acid and C-terminal acid, respectively. The parent scan mode is ideally suited for the analysis of typical fragmentation schemes and can generate spectra which contain characteristic precursors or substructures of a selected daughter ion. Microscale reactions of methionine enkephalin with phenyl isothiocyanate were performed to selectively "tag" the N-terminus for subsequent elucidation of the N-terminal amino acid.

The daughter MS/MS spectrum of the protonated molecular ion of methionine enkephalin (m/z 574) contain primarily A, B, C, and Y substructure ions (Figure 2). Fragment ions corresponding to the B, C, X, Z, and Y substructures are not present in the spectrum. The fragment ion at m/z 120 corresponds to the phenylalanine substructure and fragment ions at m/z 177 and 205 correspond to substructures of glycine-phenylalanine. The m/z 234 fragment corresponds to the glycine-glycine-phenylalanine substructure.

The daughter MS/MS spectra of the N-terminal and C-terminal sequence ions of methionine enkephalin exhibit a more selective fragmentation behavior than the spectra obtained from the molecular ion species. These spectra yield either N-terminal or C-terminal sequence ions exclusively. The fragmentation behavior of the N-terminal sequence ions of methionine enkephalin suggest that the parent MS/MS mode may be utilized to obtain exclusive N-terminal sequence information (A, B, and C substructures). Specifically, the parent MS/MS spectrum selecting all precursors of the A, substructure should provide unidirectional N-terminal sequence information. The fragmentation behavior of the C-terminal sequence ions suggest that the parent MS/MS mode may be utilized to obtain exclusive C-terminal sequence information (Y, substructures).

The parent MS/MS spectrum selecting for the precursors of the A tyrosine substructure of methionine enkephalin (m/z 136) is shown in Figure 3. All N-terminal sequence ions (A, B, and C) are present along with the protonated molecular ion. No ions corresponding to C-terminal sequence ions are present in the spectrum. These results indicate that all N-terminal sequence ions are formed in the ion source and undergo MS/MS fragmentations to yield the A, substructure ion. The B, C, and C substructures which are not present in the parent MS/MS spectrum of methionine enkephalin are readily apparent in this spectrum. The presence of the B, A, and C substructure ions indicate that these ions are indeed formed in the ion source.

The parent MS/MS spectrum selecting for all precursors of the Y, methionine substructure ion of methionine enkephalin (m/z 150) contain all the Y, substructures along with the protonated molecular ion (Figure 4). This result is due to the fact that each of Y, substructures form in the ion source and undergo MS/MS fragmentations to yield the Y, substructure ion. The complementary X, and Z, substructures are not present in this spectrum.

The parent MS/MS mode selecting precursors of the A, substructure is the most selective for generating exclusive N-terminal sequence ions. Utilization of this MS/MS sequencing methodology with amino-terminal derivatized peptides is useful for the identification of the A, substructure. The presence of all A, B, and C, fragments in the parent MS/MS spectrum indicate that each of these N-terminal sequence ions are formed in the ion source.
Figure 1. The Expected Sequence Ions of Methionine Enkephalin (Tyr-Gly-Gly-Phe-Met) Resulting From Backbone Fragmentation.

Figure 2. Daughter MS/MS Spectrum of the Protonated Molecular Ion of Methionine Enkephalin.

Figure 3. Parent MS/MS Spectrum of Methionine Enkephalin Selecting All Precursors of the A1 substructure (m/z 136).

Figure 4. Parent MS/MS Spectrum of Methionine Enkephalin Selecting All Precursors of the Y1 substructure (m/z 150).
FORMATION OF INTERNAL FRAGMENTS DURING TANDEM MASS SPECTROMETRY OF PEPTIDE [M + H]⁺ IONS

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"Internal" fragment ions of protonated peptides arise by charge retention on a portion of the structure excised from the peptide chain. The question which prompted the present studies was whether, during the formation of (BY)⁺ (or (AY)⁺) type internal fragments from peptides, the B-type (or A-type) cleavage preceded the Y'-type cleavage or vice versa. The genesis of internal fragment ions requires a minimum of two bond cleavages but the sequence of fragmentation is not revealed by a conventional product ion spectrum. Preliminary experiments1, based upon sequential product ion scanning, suggested that the Y⁺ ion from protonated leucine-enkephalin had a greater propensity to form the (B₂Y)⁺ species than did the B₂ ion. However, when two or more first generation intermediates can fragment to form the same second generation product, the relative contributions of the alternative pathways are difficult to judge based upon sequential product ion scanning alone. Reaction intermediate scanning2 can provide information regarding the relative quantitative significance of competing pathways which give rise to a common second generation product. For mechanistic studies, the population of intermediates available for subsequent decomposition during the reaction intermediate scan must be considered during the interpretation of the data. We have undertaken a broader study of the genesis of internal fragments from peptides using the reaction intermediate scanning capability of a BEqQ hybrid mass spectrometer.

Under low energy CAD conditions, leucine-enkephalin generates four internal cleavage fragments, designated as (A₂Y₂)⁺, (B₂Y₂)⁺, (A₄Y)⁺, and (B₄Y)⁺. Sequential product ion scans for four of the simple cleavage intermediate ions (B₂, A₄, Y₂, and Y₄) demonstrated that all four of the internal cleavage ions can be formed via either the B₂ ion or the Y₂ ion. However, the other simple cleavage ions also give rise to two of the internal cleavage ions, and the relative importance of these competing pathways was not apparent from second generation product ion scanning alone. In the reaction intermediate scan for the (B₂Y₂)⁺ ion from leucine-enkephalin, the major signals detected corresponded to the Y₂⁺ and B₂ ions, with the Y₂⁺ predominating. In assessing these data, reference was made to the MIKES spectrum of this peptide, which accurately represented the population of all potential intermediate ions available for subsequent decomposition during the reaction intermediate scan. The relative intensities of these two ions were reversed in the reaction intermediate spectrum as compared to the MIKES spectrum, indicating that the [M + H]⁺ → Y₂⁻ > (B Y Y)⁻ reaction sequence is much more highly favored than is the [M + H]⁺ → B₂⁻ > (B Y Y)⁻ sequence, although both reactions occur. The B₂ ion, which dominated the MIKES spectrum, also dominated the reaction intermediate spectra for the other internal cleavage ions. The relative abundances of the intermediates detected in these spectra more closely resembled those in the MIKES spectrum, indicating less dramatic differences in the relative contributions from the various competing pathways. Note that the formation of an AY ion via a B ion requires the cleavage of three covalent bonds, yet this pathway predominated for the (A₂Y₂)⁺ and (A₄Y)⁺ species.

Under low energy CAD conditions, des-Arg¹-substance P gives rise to at least eight internal cleavage ions of the A₄Y₂ and B₄Y₂ types. The metastable MIKES spectrum of this peptide (Figure 1) demonstrated that internal cleavage ions can be formed under spontaneous decomposition conditions on a microsecond time scale. In the second generation product ion spectrum representing the [M + H]⁺ → Y₂⁻ > (products) sequence for this peptide (Figure 2), all eight of the internal fragments from this peptide were detected. The reaction intermediate scan for the (B₂Y₂)⁺ ion obtained under metastable conditions (Figure 3) demonstrated that both the B₂ and the Y₂⁻ species were intermediates in the formation of this ion. Comparison with the MIKES spectrum indicated a greater inherent tendency for the Y₂⁻ species (as compared to the B₂) to give rise to the (B₂Y₂)⁺ ion. The complexity of the origins of internal cleavage fragments was further exemplified in the reaction intermediate spectrum for the [M + H]⁺ → intermediates > (B Y Y)⁻ reaction sequence for des-Arg¹-substance P. The Y₁⁺ ion was again prominent as an intermediate in this spectrum. However, all possible members of the B⁺ series were also detected, as well as several larger internal cleavage ions, and the sum total of these contributions exceeded that derived through the Y₁⁺ ion.

CONCLUSIONS: Certain Y₁⁺ ions (equivalent to truncated protonated peptides) have a high inherent tendency to fragment to (AY)⁺ (and (BY)⁺) ions. Internal fragment ions can also arise through alternative, multistep pathways which may dominate when the requisite intermediates are formed in high abundance. Reaction intermediate scanning (MS/MS/MS) reveals the relative significance of competing pathways provided the relative abundances of alternative intermediates are considered.

ACKNOWLEDGEMENT: This research was supported in part through a generous gift from Glaxo, Inc.

The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Figure 1.

Figure 2.

Figure 3.
Measurements of the kinetic energy (KE) released during the decomposition of metastable ions are conventionally performed by mass analyzed ion kinetic energy scanning (MIKES) using a BE geometry mass spectrometer [1]. However, the effective mass resolution of product ions with the MIKES technique (100-300) is insufficient to permit estimation of the kinetic energy released during the formation of two product ions which are very similar in mass and which consequently overlap in the MIKES spectrum. Furthermore, problems associated with the Derrick shift [2] can lead to erroneous interpretation even when the MIKES peaks appear to be well separated. With an EQ or a BEqQ hybrid instrument [3,4], it is possible to mass select (at unit resolution) the ions detected during the kinetic energy scan; this eliminates any uncertainty regarding the mass of the detected ions and permits deconvolution of overlapping MIKES peaks. Such a scan can thus be called a mass deconvoluted MIKES analysis (MAD MIKES). The characteristic MIKES peak shape is retained, permitting estimation of the kinetic energy released during product ion formation. Such calculations assume a single stage of bond cleavage. Caution is indicated, however, since profiles of ions generated through pathways involving sequential steps of bond cleavage can be broadened due to the kinetic energy released during the initial fragmentation, leading to potentially erroneous conclusions regarding kinetic energy release if the sequential reactions are not recognized.

We have employed the MAD MIKES technique in order to assess the kinetic energy released during the metastable fragmentation of peptide bonds. The reproducibility of the experiments has been examined, and a systematic study has been undertaken using relatively simple pentapeptides as model compounds. These experiments employed a hybrid instrument of BEqQ configuration.

RESULTS. The deconvolution of overlapping MIKES peaks through the use of the MAD MIKES experiment is exemplified in Figure 1. Peaks attributable to m/z 393 (Y4) and 397 (A4) from leucine enkephalin clearly overlap in the MIKES spectrum (upper trace), but are resolved into their individual components in the MAD MIKES spectra (lower two traces). Despite the sacrifice in signal intensity accompanying the use of MAD MIKES, careful attention to experimental parameters permits reproducible determinations of ion kinetic energy profiles, with intra-day coefficients of variation as low as 2% for calculated kinetic energy release values.

Table I summarizes the kinetic energy release calculated for metastable peptide fragmentations from MAD MIKES experiments with several simple pentapeptides. Higher values of kinetic energy release were consistently obtained from penta-Phe than were obtained for the corresponding fragmentations for penta-Gly or penta-Ala. Also noteworthy is the similarity of kinetic energy release values for B- and Y'-type ions involving cleavage of the same peptide bond (B3 and Y2 in these examples). Of particular note is the high value obtained for the A4 ion from leucine-enkephalin. A reaction intermediate scan [5] representing the [M + H]+→intermediates→A4 reaction sequence demonstrated that the A4 ion can be formed via initial formation of the B4 ion under metastable conditions. The high value observed for the kinetic energy release accompanying formation of the A4 ion is consistent with the occurrence of this pathway during the microsecond time scale of the MIKES experiment.

CONCLUSIONS. Mass deconvoluted MIKES analysis has been confirmed as an effective and reproducible technique for kinetic energy release measurements. Interpretation of kinetic energy release requires consideration of the possibility of multistage fragmentation. Several observations from these preliminary data are striking and encourage further study: (i) the kinetic energy release accompanying peptide bond cleavage appears to be influenced by the side chain structure of the amino acid constituents; (ii) an A-type ion may be formed via a B-type ion under metastable conditions on a microsecond time scale; (iii) the kinetic energy released during the formation of Y'-type ions and their B-type congeners are remarkably similar for all examples studied.

ACKNOWLEDGEMENT. This research was supported in part through a generous gift from Glaxo, Inc.

REFERENCES
Leucine Enkephalin: MIKES and MAD MIKES

Figure 1.

Table 1. Kinetic Energy Release In Peptides

<table>
<thead>
<tr>
<th>Amino Acid Sequence</th>
<th>KE Release (meV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( Y_3^* )</td>
</tr>
<tr>
<td>GGGGG</td>
<td>24</td>
</tr>
<tr>
<td>AAAAA</td>
<td>25</td>
</tr>
<tr>
<td>FFFFFF</td>
<td>29</td>
</tr>
<tr>
<td>YGGFL</td>
<td>34</td>
</tr>
</tbody>
</table>

* value consistent with the formation of the \( A_4 \) ion via the \([M + H]^+ \rightarrow B_4^- \rightarrow A_4 \) reaction sequence
EVIDENCE FOR TWO POSSIBLE MECHANISMS IN THE FORMATION OF PEPTIDE [B'_{n-1} + OH]^+ IONS

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A novel fragmentation of peptide [M + H]^+ ions has recently been described on the loss of the C-terminal amino acid residue, yielding [B'_{n-1} + OH]^+ ions [1,2]. This rearrangement was shown to occur if the C-terminus consists of a free carboxyl group and is of diagnostic importance as it allows to determine the C-terminal amino acid. Thome et al. [2] proposed a mechanism for this rearrangement based on previous research carried out by Grese et al. [3] on peptide lithium adducts.

In the course of a study on the fragmentation of [M + H]^+ ions of the heptapeptide (met^2, arg^6, phe^7) enkephalin (YGGFMRF), the [B'_{n-1} + OH]^+ ion at m/z 730 was found to be the most abundant daughter ion (Fig. 1). However, the FAB/MS/CID/MS data obtained for the corresponding \textsuperscript{18}O\textsubscript{2}-analog revealed two corresponding ions at m/z 732 and 734 (ratio 3/1), for which only the former can be explained with the mechanism proposed by Thome et al. [2]. These data indicate that a single mechanism does not apply to this rearrangement. In an effort to rationalize the ions at m/z 734 in the \textsuperscript{18}O\textsubscript{2}-analog, a second mechanism is proposed (Scheme 1). This mechanism results in [C'_{n-1} + COOH]^+ ions, which in the case of the unlabelled product fall at the same mass as the [B'_{n-1} + OH]^+ ions.

An interesting feature is that this C-terminal rearrangement occurs in a peptide containing an arginine residue at the n-1 position, for which the formation of these rearrangement ions was shown to be inhibited [2,3]. Lowering the basicity of the arginine residue by conversion to its dimethylpyrimidylornithyl derivative resulted in a decrease of the abundance of the rearrangement ion.

REFERENCES


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FIGURE 1
FAB/MS/CID/MS spectrum obtained for [M + H]^+ ions of the heptapeptide (met^5, arg^6, phe^7) enkephalin showing an abundant [B'_{n-1} + OH]^+ ion at m/z 730.

SCHEME 1
Mechanism proposed for the formation of [C'_{n-1} + COOH]^+ ions.
Fast atom bombardment (FAB) with collisionally activated dissociation (CAD) of peptides yields a number of sequence ions that provide information about the primary structure. Specific mechanisms have been suggested and are widely accepted for the most commonly detected sequence ions: \( a_n \), \( b_n \), \( c_n \), and \( y_n \). However, few stable isotope labelling studies have been performed to substantiate these proposed mechanisms.

We previously described a new method for localizing a positive charge on either the N- or C-terminus of a peptide by derivatizing the peptide with an ethyl-triphenylphosphonium moiety. The FAB-CAD-B/E (linked scan) mass spectra of both the N- and C-terminal ethyl-TPP derivatives indicate ions are formed that differ in mass from the corresponding fragment ions in the spectra of the derivatized peptides by one to three mass units (not including the difference in mass due to the derivatizing moiety); we interpret these differences to be reflective of the formation of these fragments via charge-remote fragmentation mechanisms, rather than via charge-directed processes.

Using two different stable-isotope-labeled analogues of the peptide VGVAPG, we have investigated the mechanism of formation of some of these fragment ions. One of the analogues contained a deuterium on the \( \alpha \)-carbon of the alanine; the second deuterated analogue contained deuteriums at all exchangeable sites \((-\text{COOH}, -\text{CONH}_2, -\text{NH}_2)\) of the peptide (figure 1). The peptide VGVAPG, the deuterated labeled peptides, and the N- and C-terminal ethyl-TPP derivatives of the unlabeled and deuterium labelled peptides were analyzed by FAB-CAD.

The formation of \( y_n \) and \( c_n \) fragment ions is thought to involve protonation during ionization and the transfer of a second hydrogen atom to the amide nitrogen during bond cleavage. The hydrogen to be transferred to the amide involved in the bond cleavage can originate from several sites: (1) the \( \alpha \)-carbon, (2) an \(-\text{NH}_2\), or (3) one of the side chains.

The data indicate that both ions are formed by transfer of a hydrogen from an amide group. If the hydrogen transfer is from the adjacent amide, this would explain the absence of \( y_n \) ions that are adjacent on the C-terminal side to a proline residue, which does not contain a H on the imide group. Interestingly the spectrum of N-acetylated VGVAPG is noticeably different; the intensities of the \( y_n \) ion peaks are reduced dramatically from those of the peptide. This suggests that the N-terminal free amine may be the primary source for the hydrogen transferred during the formation of the \( y_n \) fragment ions.

Because CAD fragment ions from the ethyl-TPP derivatives of peptides are not protonated at the site of cleavage, but have a fixed positive charge, the structures and mechanisms for their formation were expected to differ from those of the derivatized peptides. We have found that the C-terminally derivatized peptide produces \( y_n-3 \), \( x_n-3 \), \( z_n \), \( v_n \), and \( w_n \) ions, while N-terminally derivatized peptides yield \( a_n-1 \), \( d_n \) and, to a lesser extent, \( b_n-1 \) and \( c_n-1 \) fragment ions. We speculate that the mechanism for forming the ions that are either 1 or 3 mass units lower than expected (compared to those from the derivatized peptides) does not involve protonation or a hydrogen transfer, but involves a 1,2 elimination reaction or the expulsion of a molecule of hydrogen. The expelled H atom can originate from three possible sites: (1) the \( \alpha \)-carbon, (2) an amide nitrogen or another exchangeable site, and (3) the \( \beta \)-carbon or the sidechain of the amino acid involved in the cleavage. Using the N- and C-terminal derivatives of the deuterated analogues of VGVAPG, the locations of the expelled H atoms from the molecular ion to form \( y_n-3 \), \( x_n-3 \), and \( a_n-1 \) fragment ions were determined.

From the mass spectra of the C-terminal ethyl-TPP derivatives of the deuterated analogues, it was deduced that formation of the \( y_n-3 \) ion proceeds through the loss of a H atom located on the \( \alpha \)-carbon. Scheme 1 represents a possible mechanism for formation of the \( y_n-3 \) ions. Similarly, it was found that \( x_n-3 \) ions form through expulsion of a H atom located on an amide N, represented in scheme 2 as a 1,2 elimination reaction. The mass spectra of N-
terminal ethyl-TPP derivatives of the deuterated analogues indicate that the $a_{1\text{n}}$ ion is formed by the loss of neither the H atom on an amide nor that on the $\alpha$-carbon. This suggests that the $a_{1\text{n}}$ ion is formed by loss of a H atom from the $\beta$-carbon located on the side chain of the amino acid involved in the cleavage. This mechanism, represented in scheme 3, suggests a 1,2 elimination reaction. We do not expect the fragmentation to involve expulsion of a hydrogen molecule from the molecular ion, since the $a_{1\text{n}}$ could not occur at residues that are $N$-terminally adjacent to a proline by this mechanism, and the spectra do indicate these ions. In addition the 1,2 elimination mechanism explains the absence of $a_{1\text{n}}$ ions for cleavages involving a glycine residue, as it does not contain a hydrogen-bearing carbon side-chain.

References
Protein Conformational Correlations in The Solution State and The Gas Phase: An Ionspray and Collision Activation Study

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It has been shown\(^1\) that the charge distribution profiles of protein ions generated from electrospray ionization are sensitive to the protein conformational changes in solution. Thus, it can be used to characterize protein folding properties. However, it remains unclear whether these conformational states still maintain their structural differences in the gas phase, where no or little solvation assistance is available. Addressing this question is of fundamental importance to our understanding of the nature of protein folding.

Two conformational states of papain (23,422 Da) in aqueous solution were detected using an ionspray (pneumatically assisted electrospray) mass spectrometer (Sciex API III MS). The first state (N state), whose charge distribution profile centered around 11+, probably corresponded to the native conformation, as it was the only charge profile detected in the neutral solution (Fig. 1a). The second state (D state) (Fig. 1c), which co-existed with N state in 10% acetic acid solution (Fig. 1b), had a profile centered around 19+, indicating a more extended structure, as the denatured protein had exposed more basic amino acids for charging. Upon further increasing the acidity (to 20% acetic acid), N state disappeared and only D state profile remained (Fig. 1c).

The ions from the two co-existing conformational states in solution were subjected to collisions in the gas phase to probe for their structural differences. The collision experiments were carried out in either the ionspray interface region with N\(_2\) collision gas or the MS/MS collision chamber with Ar gas in Q\(_2\) region of the triple quadrupole MS. Although the N state profile showed much less change upon collisions, the D state profile disappeared quickly with the increase of either the collision energy in the interface region (Fig. 2) or gas density in Q\(_2\) (Fig. 3), presumably due to the fragmentation or scattering loss of the D state ions. A clear discontinuity in the intensity pattern existed in the cross-over region of the two profiles, where the ions from the two conformational states have similar number of charges. Thus, the velocity difference between the low- and high-charged ions was not the major cause for their behavioral difference during collisions.

The observed collisional phenomenon demonstrates that the protein conformational difference still exists in the gas phase, which in turn implies that solvation is not a necessity for a protein to maintain a certain conformation. The following factors may account for the gas phase behavioral difference of the two conformational states. First, the compact ball-type conformation of N state has smaller physical cross-section than, and therefore has less scattering loss than, the extended conformation of D state. Second, the tight structure of N state may have wrapped the majority of the protein backbone tightly within, and the scission of the bonds on the ball surface may not lead to the release of the resulting fragments. Consequently, N state appears less sensitive to collisions, as the limited fragmentation has not changed its effective overall mass. On the contrary, the loose structure of D state enables the fragmented backbone pieces to fly away, thus resulting in the quick decrease in its profile intensity. Since the extended D state has a larger cross-section than the tightly folded N state, it may also on average encounter more collisions than N state, which in turn leads to more energy intake and more fragmentation for D state.

Similarly, two conformational states, i.e., the low-charged N state and the high-charged D state, of myoglobin (16,950 Da) were detected in neutral solution (Fig. 4a) (in acidic solution only D state is stable). In contrast to the two papain states that have the same mass, the mass of myoglobin N state is 616 Da higher than that of D state, due to the trapping of the non-covalently bound heme in N state. As in case of papain, ion-neutral collisions in Q\(_2\) region resulted in the disappearance of myoglobin D state, while N state was relatively less affected (Fig. 4b). However, further increase in collision gas density resulted in the gas phase denaturation of N state, as there appeared a new charge profile (Fig. 4c) in which heme had been collisionally removed (the protein mass is now 616 Da less than before).
Myoglobin, which has a linear structure with no disulfide cross linkages, has to have a native conformation to wrap heme inside. The fact that its N state can hold heme in the gas phase, and is relatively more resistive to collisions than its D state, indicates that N state is very likely to have retained the native conformation during the flight in vacuum. This N state also appears to be flexible in the gas phase, as indicated by the collision denaturation experiment (As a comparison, the conversion of myoglobin N state to D state in solution is also very fast). On the contrary, papain N state appears to be rather rigid in the gas phase, paralleling its high stability in solution.

The structural and stability correlations of the compact and the extended protein conformations in the solution state and the gas phase indicates that the isolated gaseous protein molecules, without the assistance of the solvation effects, are capable of retaining the conformational identities they have in solution.

Reference:
CHARACTERIZATION OF VARIOUS DERIVATIVES OF CYSTEINE-CONTAINING PEPTIDES USING HYBRID TANDEM MASS SPECTROMETRY

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Modifications of cysteine residues are implicated in the modulation of the biological properties of proteins. In the case of low density lipoprotein, which is the major carrier of cholesterol in the blood, modification of its protein component (apoprotein B-100) under oxidative conditions is known to influence cholesterol accumulation in the cells. Cysteine residues might be modified under these conditions. As an initial stage in the development of an analytical strategy to assess such changes, the synthetic nonapeptides, GTYGLSCQR and EELCTMFIR, corresponding to the tryptic fragments of apoprotein B-100 from the residues 1472 to 1480 and 4187 to 4195 respectively, were analyzed by tandem mass spectrometry.

Low energy ($E_{\text{lab}} = 8-13 \text{ eV}$) collisionally activated decomposition (CAD) of the protonated peptides resulted in a low fragmentation yield and gave poor product ion spectra displaying only a few $Y^\nu$ ions.

Derivatization of the cysteine residues of these nonapeptides was attempted to increase the sensitivity of detection and optimize the MS/MS fragmentations. Carboxymethylation of the thiol moiety was performed by initial treatment of the peptide with a 10-fold molar excess of dithiothreitol (DTT) and then adding a 50-fold molar excess of iodoacetic acid, sodium salt over DTT. Carboxymethylation of the cysteine residue gave only a minor improvement of the MS/MS fragmentation yield of the native peptide.

Modification of the nonapeptides under oxidative conditions was then performed by reacting the peptides with a 100-fold molar excess of freshly prepared performic acid. Conversion of the cysteine residues to cysteic acids and of the methionine residue to methionine sulfone resulted. The MS/MS analyses of these oxidized forms of the peptides under low energy CAD conditions gave product ion spectra displaying the complete series of $Y^\nu$ ions. The marked simplification of the product ion spectra allowed an easy determination of the localization of the oxidation on the tryptic peptide sequence. Moreover, the fragmentation yield was dramatically improved compared to the native peptides, implying intra-ionic interactions involving the cysteic acid residue.

To observe further the effect of converting cysteine residues to their cysteic acid forms, another synthetic tryptic peptide was studied: GFLCGHYR. The oxidized form of the peptide again gave an improved MS/MS fragmentation yield under low energy CAD compared to the native peptide and produced the complete series of $Y^\nu$ ions (Figure). High energy CAD (MIKES) analyses of the native peptide and its oxidized form gave fragmentations similar to those observed using low energy CAD (several $w$ ions were additionally observed in the MIKES spectra). A higher fragmentation yield of the oxidized form of the peptide was also observed under high energy CAD.
Low energy CAD of \((M+H)^{+}\)

Native GFLCGHYR

Low energy CAD of \((M+H)^{+}\)

GFLC*GHYR (* Cysteic acid form)

Low energy CAD of \((M+H)^{+}\)
DETERMINATION OF AMIDE HYDROGEN EXCHANGE RATES IN PEPTIDES BY CONTINUOUS-FLOW FABMS

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Hydrogen exchange has been used in conjunction with FABMS for a variety of structure elucidation problems. Sethi et al. (BBRC 112, 126-131 (1983)) demonstrated that the number of rapidly exchanging hydrogen atoms can be determined if glycerol d$_3$ is used as the matrix. The amide hydrogens in peptides exchange more slowly and are not included in the group of rapidly exchanging hydrogens. Since the rate at which amide hydrogen atoms undergo exchange may be a useful probe of the structures of peptides and proteins, we have developed a method based on continuous-flow FABMS for determining their rates of exchange.

Although NMR has been the principal method for determining amide hydrogen exchange rates, it has disadvantages associated with peptide size, and solubility, and with the quantity of peptide required for measurements. CF FABMS offers advantages in all of these areas. Results obtained by CF FABMS and NMR for two model peptides are presented here.

Exchangeable hydrogens can be classified as either slow or fast, depending on their location in a peptide. These two classes of hydrogens are illustrated for bradykinin in Fig. 1. Amide hydrogen exchange is base catalyzed, and occurs approximately 1000 times faster at pH 8 than at pH 3. Slow and fast exchanging hydrogens in peptides were fully exchanged with deuterium by dissolving the peptides in deuterated solvents at pH 7 and 20°C. After approximately 1 hour, this solution was diluted 20 times with H$_2$O/acid (pH 2-3). The temperature was reduced to 0°C to reduce the rate of back exchange. Under these conditions, rapidly exchanging hydrogens revert back to protium within seconds, while slowly exchanging (amide) hydrogens may require a few hours to undergo exchange. The back-exchange of amide deuterium to hydrogen was followed by CF FABMS using a continuous-flow probe that has been described previously (1,2). To reduce the likelihood of back-exchange during the analysis, the injector and most of the fused silica was submersed in an ice bath. The first injection was made within 1 minute after addition of H$_2$O, with additional injections made at regular intervals for at least one hour. Mass spectra were acquired with a Kratos MS-50/DS-90 system and transferred to a Macintosh computer where the number of deuterium atoms per peptide was calculated. Part of this calculation required adjusting intensities for contributions from naturally occurring isotopes.

The average rate constant for hydrogen exchange, 2.2 x 10$^{-4}$ was determined by plotting the logarithm of the deuterium content vs time (Fig. 2). Data from replicate experiments indicate that the rate for back exchange can be determined with a coefficient of variation of 14%. The rate of hydrogen exchange in this peptide was also determined for the same conditions by monitoring the amide hydrogen signals by 500 MHz NMR. Data from this experiment, plotted in Fig. 3, show that essentially the same value for back-exchange was obtained by this alternate method.

Results presented here demonstrate that the rate at which amide hydrogens undergo isotopic exchange can be determined accurately by direct-injection FABMS. For slow exchanging conditions, pH 2-3 and 0°C, the half-life for exchange is 53 minutes. Since proteolytic digests of proteins can be analyzed in less than 20 minutes by directly-coupled HPLC FABMS, it follows that the number of amide hydrogens that have undergone exchange with deuterium can be determined by this approach.


Figure 1  Structure of bradykinin illustrating hydrogen atoms that undergo rapid O and slow N isotopic exchange.

Figure 2  Kinetic plots illustrating the loss of deuterium from the fragment β1-14 of the peptic digest of hemoglobin determined by CF FABMS.

Figure 3  Kinetic plot illustrating the loss of deuterium from the fragment β1-14 of the peptic digest of hemoglobin determined by NMR.
EFFECT OF MASS SPECTROMETRIC MATRIX SOLVENT ON THE KINETICS OF PEPTIDE HYDROLYSIS BY CARBOXYPEPTIDASE-Y

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The use of enzymes in mass spectrometry has found both on-line and batch sampling applications. In conjunction with various mass spectrometric sampling techniques, either approach can greatly facilitate sequencing of biomaterials, especially peptides. The most common application in this context is predigestion of protein analytes with specific endopeptidases, generating a mixture of smaller peptides amenable to sequencing analysis, usually by fast atom bombardment (FAB). This has proven to be a remarkably powerful sequencing approach, requiring relatively little material to provide, in favorable cases, full primary sequence information.

On-line applications offer the distinct possibilities of real-time monitoring of enzymatic reactions proceeding in situ within the spectrometer source or sampling system. This can in principle provide kinetics information (rates and mechanisms) in addition to relatively more limited sequencing. Limitations arise from the requirements of the various mass spectrometric sampling approaches which have been successfully employed, including thermospray, flow-FAB, and electrohydrodynamic (EH) MS. Successful applications must balance the chemical requirements of the reacting system with those necessary for stable and sensitive MS sampling, while achieving reaction on a time scale compatible with MS scanning. Under the best of cases, the MS data can provide an indication of reaction rates and intermediates. The latter information can provide useful mechanistic insight even if solvent modifications necessary for MS compatibility enforce significant deviations from the purely aqueous solvent conditions generally of greatest interest for studies of enzyme kinetics.

In one such application, we have used EH MS to monitor the progress of peptide hydrolysis proceeding in situ within the ion source, under the influence of the exopeptidase Carboxypeptidase-Y (CPY). For the pentapeptide leucine enkephalin (YGGFL), the procedure allowed observation of the disappearance of the intact substrate, plus the appearance and disappearance of each of the two reaction intermediates (YGGF, then YOG), and the generation of the final products (YG, G, F, and L). The kinetics curves had generally the correct qualitative appearance, but the organic co-solvent thiodiethanol (TDE), required for reasonably stable and sensitive EH sampling, slowed the rate of the reaction so that almost 20 hours were required for the reaction to “go to completion.” Because MS sampling was only active intermittently, sample consumption was limited to about 300 μL of 18mM solution (6 μmol, total sample consumption). In comparison with other peptide MS experiments, this is rather high; the specialized role of this approach for cases where kinetics or C-terminal information is of interest is thereby evident.

In order to assess the effect of the co-solvent on enzyme kinetics and MS sensitivity, we have undertaken parallel optical and EH mass spectrometric kinetics experiments, evaluating the rates of hydrolysis of selected dipeptides in matrices comprised of various TDE/water mixtures, plus phosphate buffer (pH 6.5, total phosphate 12.5 mM) and 0.5 M NaCl supporting electrolyte. For the simple dipeptide, two approaches to interpretation of the kinetics data are possible. At a given enzyme and substrate concentration (with substrate in excess), the rate of amino acid appearance or dipeptide disappearance can be modeled as a (pseudo-) first order reaction. An exponential fit of concentration vs time data can therefore provide an estimate of enzyme activity as represented by the first order rate constant, k. Repetition of the experiment at a variety of substrate concentrations (fixed [CPY]) can provide two additional measures of activity: the Michaelis constant (K_m, related to the substrate concentration at which the enzyme becomes saturated) and the maximum rate (V_max) at which hydrolysis proceeds in the presence of excess substrate. Values for these parameters derived from optical data are summarized in Table I, which shows that as little as 10% TDE attenuates enzyme activity by as much as 32%. For the concentrations employed, the hydrolysis took roughly 45 min.
Unfortunately, the MS limit of detection was significantly lower than that for the optical experiment, so that much higher enzyme and substrate concentrations were necessary for MS monitoring. However, it should be feasible to measure a pseudo-first order rate constant in reasonable agreement with that determined from standard optical experiments. This would be a promising indication that the kinetics in the presence of co-solvent are accurately represented by the MS.

This result gains significance when considering hydrolysis of a more complex, polypeptide substrate. For hydrolysis of the pentapeptide leu-Enk, the optical method is incapable of distinguishing contributions from the two intermediates and the final reaction products. Instead, the absorbance data (Figure 1) represent a convolution of the three sequential reactions. By contrast, in the MS data, contributions can be easily resolved, so that determination of the kinetics parameters for each separate step should be readily feasible. Figure 2 summarizes the time dependence of the EH mass spectrum of Leu-enkephalin, showing the variations in abundance of each residue (Figure 2a) and each amino acid final product (Figure 2b).

References

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Table 1. KINETICS PARAMETERS for HYDROLYSIS of CBZ-Phe-Leu by CPY

<table>
<thead>
<tr>
<th>Matrix</th>
<th>[Enzyme] (units/ml)</th>
<th>k (min⁻¹)</th>
<th>Kₘ⁻</th>
<th>Vₘ₉₉ (mM/min)</th>
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</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>0.1</td>
<td>0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O + NaCl</td>
<td>0.1</td>
<td>0.28</td>
<td>0.087</td>
<td>0.096</td>
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<td>10% TDE</td>
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<td>0.207</td>
<td>0.060</td>
</tr>
<tr>
<td>20% TDE</td>
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<td>0.12</td>
<td>0.329</td>
<td>0.024</td>
</tr>
<tr>
<td>40% TDE</td>
<td>0.1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40% TDE</td>
<td>0.5</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Pseudo-first order rate constant.  † Michaelis constant.
† Maximum rate for saturated enzyme.

Fig. 1 Optical monitoring of Leu-Enk hydrolysis (540nm). [CPY] = 1 unit/ml; [Leu-Enk] = 0.1 mM; 20% TDE

Fig. 2. MS monitoring of Leu-Enk hydrolysis. [CPY] = 2.2 units/ml; [Leu-Enk] = 18 mM; 60% TDE

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Alkaline earth metal ions form anionic complexes with di- and tripeptides upon FAB ionization. Two such complexes of the elemental composition, \([\text{pept} + \text{Met}^2+ - 3\text{H}^+]^{-}\) and \([2\text{pept} + \text{Met}^2+ - 3\text{H}^+]^{-}\), were studied by tandem mass spectrometry.

The CAD spectra of \([\text{pept} + \text{Met}^2+ - 3\text{H}^+]^{-}\) are very simple with respect to the spectra of protonated peptides and metal ion cationized peptides. The structure of this anion was shown to be one in which the metal ion binds to the deprotonated C-terminal carboxylate group and to two amide nitrogen atoms. Supporting evidence is that peptides without free C-terminal or amide hydrogens on the middle amino acid do not form this anionic ion. Fragmentation of labelled peptides shows that both remaining active hydrogen atoms are located at the N-terminal. This tightly bound complex has a free or loosely bound N-terminal amino acid. Upon CAD fragmentation it can be expected to be at N-terminal because it would not be productive to break bonds at other sites. The two abundant fragments, \(F_1\) and \(F_2\) are the products of decomposition of the N-terminal acid residue(spectra A and B and Scheme 1). The approach can be used to determine N-terminal amino acid.

The metal ion in \([2\text{pept} + \text{Met}^2+ - 3\text{H}^+]^{-}\), on the basis of experiments with C-terminal blocked peptides(for example, peptides with an ester or amide at C-terminus do not form this complex), binds to the C-terminal carboxylate groups of both peptides. The third binding site is the amide nitrogen atom of the C-terminal amino acid, which is supported by the experiments with proline containing peptide, that is, Ala-Pro does not form this anion whereas Ala-Pro produces this complex extensively and the complex fragment regularly.

The CAD spectra of \([2\text{pept} + \text{Met}^2+ - 3\text{H}^+]^{-}\) show redundant sequence information(spectra C and D and attached schemes). The peptide sequence can be read from the y-type fragments; w-type ions are abundant if the amino acid that has the potential to form a w-type ion is not an N-terminal amino acid(spectrum c, the ion of \(m/z\) 325). Leucine and isoleucine can, therefore, be differentiated by the abundant w-type ions in the spectra. The CAD spectra of metal-bound dimers of peptides may be useful in peptide sequencing and isomer differentiation.

Another abundant fragment ion in the spectra of \([2\text{pept} + \text{Met}^2+ - 3\text{H}^+]^{-}\) complexes is the \([\text{pept} + \text{Met}^2+ - 3\text{H}^+]^{-}\) ions(the ion of \(m/z\) 239 in spectrum C and \(m/z\) 316 in spectrum D). The fragmentation process is illustrated in Scheme 4. The major evidence includes that \([\text{pept} + \text{Met}^2+ - 3\text{H}^+]^{-}\) complexes formed from tripeptides with proline at the middle do not produce \([\text{pept} + \text{Met}^2+ - 3\text{H}^+]^{-}\) ions, indicating the amide hydrogen of the center amino acid is necessary in the fragmentation. The favourable formation of \([\text{pept} + \text{Met}^2+ - 3\text{H}^+]^{-}\) reflects the stability of this complex.

The interaction of negatively charged peptides and alkaline earth metal ions appears to involve the carboxylate at the C-terminus and secondly deprotonated amide nitrogens. This conclusion applies to both metal ion bound anionic peptide complexes and metal ion bound anionic peptide dimers. We intend to pursue the mechanisms of fragmentation of these complexes and their applicability in sequencing and structure proofs.
Mass Spectrometric Analysis of Modifications to α-Crystallins

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The transparency of the lens depends on the proper close packing of the structural proteins of the lens. Posttranslational modifications to these proteins, called crystallins, can lead to formation of cross-links and aggregates that disrupt their native three-dimensional structure. We have used FABMS to examine two types of modifications to lens α-crystallins that are associated with cataractogenesis. The first, carbamylation, is a posttranslational modification that may occur in patients with chronic diarrhea or kidney failure due to a reaction of lysyl residues with isocyanate. The cataractogenesis of this modification is supported by Harding and Rixon's study showing that homocitrulline, the product formed by acid hydrolysis of carbamylated lysines, is present in cataractous Pakistani lenses. The other modification is the reaction of ascorbic acid with α-crystallin, a reaction that may be responsible for the development of senile cataract. A proposed mechanism for cataract due to ascorbic acid suggests that an oxidized form of ascorbic acid reacts with lysines of the crystallin causing further reaction and the formation of cross-links. The specific sites at which isocyanate and ascorbic acid react have not been determined. We have incubated isolated α-crystallins in vitro to cause these modifications and have identified the reactive residues and the extent of modification.

Bovine lenses were homogenized in 20 ml buffer (0.5 M NaCl, 0.001 M EDTA, 0.05 M Tris, pH 7.4), centrifuged to remove particulates, and fractionated into α-, β- and γ-crystallins by gel filtration chromatography (Sephadex G-200, 60x5 cm column, 10 ml/hr).

For the carbamylation study, the α-crystallins from the gel filtration were separated into αA- and αB-crystallins by reversed phase HPLC. αA, which was identified by electrospray ionization mass spectrometry, was used in the carbamylation experiments. A portion of the αA-crystallins was incubated in 0.1 M KCNO, 0.2 M NH₄OAc, pH 7.2, 0.02% NaN₃ at 37 °C for time intervals of 1-70 hours. A control of αA-crystallin incubated in 0.1 M KCl was prepared.

For the ascorbic acid study, α-crystallins from the gel filtration were incubated in 20 mM ascorbic acid with 40 mM NaCNBH₃ for one week at 37 °C and then separated by reversed phase HPLC. In both studies, the modified and unmodified proteins were digested with trypsin or chymotrypsin (1:50 enzyme:substrate in 0.1 M Tris, pH 8.2, at 37 °C for 1 hr) or with pepsin (1:50 enzyme:substrate in 0.03 N HCl, at 37 °C for 1 hr). The enzymatic digests were fractionated by reversed phase HPLC.

The peptides present in each fraction of the HPLC separated digests were identified by fast atom bombardment mass spectrometry (FABMS) using a Kratos MS-50 RF mass spectrometer. Portion corresponding to all of the sequence of unmodified α-crystallin was used in the carbamylation experiments. A portion of the αA-crystallins was incubated in 0.1 M KCNO, 0.2 M NH₄OAc, pH 7.2, 0.02% NaN₃ at 37 °C for time intervals of 1-70 hours. A control of αA-crystallin incubated in 0.1 M KCl was prepared.

For quantitation of the extent of modification, the pair of modified and unmodified peptides was collected in the same fraction and analyzed by FABMS. Investigations with model peptides comparing UV absorbances and FABMS intensities indicated that the FABMS responses for carbamylated and uncarbamylated peptides were similar. The relative intensities of modified and unmodified peptides, averaged over several scans, were used to calculate the percent modification at each lysine for incubations of 1, 2, 4, 8, 18 and 70 hours (Fig. 1). The extent of modification of each lysine after incubation of α-crystallin in ascorbic acid for 1 week is shown in Table 1.

The present study demonstrates that FABMS gives more specific and accurate information about the identification and quantitation of posttranslational modifications to lens crystallins than other methods. This precise information should prove valuable in assessing the factors, such as nearby sequence and conformation, which affect the reactivity of the specific lysyl residues. For bovine lens α-crystallins, the similarity in the reactivity of some lysine residues to carbamylation by isocyanate and glycation by ascorbic acid suggests that the three dimensional configuration is a major determinant of reactivity. Because human and bovine lens crystallins have similar sequences, the techniques developed in this study can be easily extended to study of human cataractous lenses. Such information about the modifications to lens proteins will be useful in designing drugs for the prevention and treatment of cataract.
Figure 1  Percent carbamylation of lysines in \( \alpha \)-crystallin

Table 1. Mass Spectrometric Analysis of Ascorbic Acid
Modification to \( \alpha \)-crystallin

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Measurement of the Velocity Distribution of Polypeptide Ions Produced by Matrix-Assisted Laser Desorption

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1230 York Avenue, New York, NY 10021

This poster reported the measurement of the velocity and translational kinetic energy distributions of polypeptide ions produced by matrix-assisted laser desorption. These distributions were determined for ions that were produced in a field-free region of the ion source, removing all effects on the velocity distribution caused by ion extraction fields. The measurement was also mass resolved: the velocity distributions of different ions produced by the laser desorption event were simultaneously obtained.

In order to measure the velocity distribution of the ions, two experiments were performed. The first experiment measured the transit time of a particular ion species through the time-of-flight mass spectrometer with a high extraction field in the ion source. The second experiment measured the transit time of the same ion species, without any initial extraction field, that is with a field-free drift region prior to the high voltage acceleration field. These two measurements allowed the calculation the ion velocity distribution intrinsic to the ion production/sublimation process by calculating the velocity of ions crossing the field-free region used in the second experiment.

Three different polypeptides were examined: angiotensin (MM = 1030 u); insulin (MM = 5730 u); and superoxide dismutase (MM = 15,590 u). Each sample was prepared by mixing one microliter of a 100 micromolar solution of the polypeptide with five microliters of a 50 millimolar solution of sinapic acid (3,5-dimethoxy-4-hydroxy-(trans)cinnamic acid, obtained from Aldrich). One microliter of this mixture was placed onto the mass spectrometer's probe tip and dried. The sample was then inserted into the mass spectrometer and used to perform the two experiments described above.

The laser desorption ion source consisted of a series of parallel acceleration electrodes and an einzel lens. The laser used was a Lumonics HY400 Nd:YAG, operated in the Q-switched mode. The laser pulse duration was approximately 10 ns. The fundamental frequency was tripled, resulting in a wavelength of 354 nm. The laser fluence used to produce the matrix-assisted laser desorption event was 10 - 15 mJ/cm² (irradiance = 1 - 1.5 MW/cm²). The result of these experiments was the discovery that all three polypeptide molecules had very similar initial velocity distributions, independent of the molecule's mass. All three molecules had distributions centered at approximately 750 m/s. Therefore, the translational kinetic energy distributions for these molecules scaled directly proportional to the molecular mass according to the following expression:

\[
KE_{av} \text{ (eV)} = 0.003 \times \text{mass (u)}.
\]

This result has consequences for theoretical models proposed to explain the matrix-assisted desorption effect and for the design of ion sources and mass analyzers. Ion sources with low ion trapping potentials, such as ion cyclotron resonance mass spectrometers, will show strong mass discrimination effects caused by the high ion velocities intrinsic to the desorption event.
Covalent attachment of long chain saturated fatty acids has been recognized as an important post-translational modification of a wide range of proteins, and is considered essential for the proper functioning of these proteins in vivo. Fatty acids most commonly observed attached to proteins are myristate, bound to N-terminal glycine via an amide bond, and palmitate, linked to cysteine residues through a thioester bond. Characterization of the nature of the fatty acid moiety using mass spectrometry has proved useful in elucidating the presence and site of binding of such modifications. The objectives of this research are to gain an understanding of the fragmentation behavior of fatty acylated peptides to develop a general methodology for sequencing and determining the specific fatty acyl group and its binding site. In this investigation, model peptides and amino acids were reacted with myristoyl and palmitoyl acid chlorides to yield both mono-, di-, and per-acylated derivatives, and their fragmentation behavior investigated using FAB ionization and MS/MS techniques.

Monoacylated samples were prepared by first dissolving the amino acid or peptide in a pH 6.0 MES buffer and adding the acid chloride as a 300 mM solution in THF. The sample was incubated with agitation at 37°C for two hours. The reaction products were then isolated on a C18 solid phase extraction cartridge (Bond Elut), rinsed with 0.01 M sodium bicarbonate followed by DI water and then eluted with methanol. Multiply acylated amino acids and peptides were prepared and isolated in a similar manner, except a pH 9.5 borate buffer was used.

Positive ion mass spectra were obtained on a VG ZAB-HS-2F mass spectrometer using FAB ionization. The protonated molecular ions were further analyzed by CAD/MIKES, using helium as the collision gas. Both dimyristoylated and dipalmitoylated lysines showed loss of the fatty acid moiety as a ketene (R-CH=C=O) to yield the major fragment ion (Fig. 1). In addition, a minor fragmentation pathway for both dimyristoylated and dipalmitoylated lysines appears to involve loss of RCONH₂, whereas the monomyristoylated lysine showed only loss of the fatty acid as a ketene. The positive ion CAD spectrum of N-acetyl-S-palmitoylcysteine ([M+H]+ = 402) similarly shows loss of the palmitoyl residue as a ketene by cleavage of the thioester bond, producing a characteristic ion at m/z 164. Additionally, the [M+Na]+ = 424 also produced a series of less intense ions 14 mass units apart, indicative of the palmitoyl moiety, resulting from charge remote fragmentation.

In contrast to the amino acids studied, fragmentation of the monomyristoylated peptide Lys-Tyr-Lys ([M+H]+ = 648) did not result in preferential loss of the fatty acid moiety as a ketene, rather a prominent A₁ fragment was observed at m/z 311, in addition to loss of the fatty amide ([CH₃(CH₂)₁₄CONH₂] at m/z 421 (Fig. 2). The sodiated adduct of monomyristoylated Lys-Tyr-Lys ([M+Na]+ = 670) was also observed. The CAD/MIKE spectrum of 670 showed the most prominent ion as 542, corresponding to loss of the C-terminal amino
acid, typical for sodiated peptides, but provided little useful information characterizing the bound fatty acid. In summary, fragmentation of [M+H]+ ions of fatty acylated peptides and amino acids often yields an intense daughter by neutral loss of fatty ketenes or fatty amides. Further experiments will test how the proximity of the site of protonation influences this behavior.

References
1.) James, G. and Olson, E N., (1990) Biochemistry, 29(11), pp 2623-2633.
Protein Complex of Rabbit Lung Flavin-containing Monooxygenase and Calreticulin Discovered by Mass Spectrometry

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²Dept. of Food Science and Technology, Oregon State University, Corvallis, OR 97331

Flavin-containing monooxygenase (FMO) is an important mammalian monooxygenase that catalyzes the NADPH-dependent oxygenation of nitrogen-, sulfur-, and phosphorous-containing drugs and pesticides in many tissues. The FMO isolated from rabbit lungs displays remarkable differences from the hepatic enzyme in catalytic behavior and sensitivity to thermal inactivation. The primary sequence of FMO from rabbit lung deduced from cDNA data shows more than 50% homology to the liver FMO. The difference in the primary sequence does not seem to be adequate to account for the enzymatic differences between lung and liver enzymes. There is also no information available concerning post-translational modification of these proteins. In this study, the lung FMO was subjected to denaturation, S-S bond reduction, carboxymethylation, digestion with endoproteinase Glu-C and trypsin, and mass spectrometric analysis. A peptide molecular-weight map which covered more than 90% of rabbit lung FMO's sequence was generated. The N-terminus and several peptides which contain FAD and NADP⁺ binding sites were sequenced by tandem mass spectrometry. The N-terminus was blocked with an acetyl group, similar to hog liver FMO*. In the process of mapping rabbit lung FMO, we found more than 50 peptides whose molecular weights did not correspond to the FMO sequence. This suggested the presence of another protein tightly associated with FMO. Since the proteinase digests generated very complicated peptide mixtures, each of HPLC fractions usually contained several peptides (see Fig. 1). It was difficult to sequence these peptides by conventional Edman sequencing. Several of the unidentified peptides were subjected to tandem mass spectrometric sequencing. Analysis by tandem MS generated sequences for 70% of those peptides (see an example in Fig. 2). Those sequences were searched for the protein data base which showed a 100% match with a calcium binding protein, calreticulin. This protein is not a simple contaminant of the lung FMO preparation because of the large difference in properties between the two. Calreticulin apparently complexes and solubilizes the FMO. The exact physiological role of both the FMO and the calcium binding protein are currently under investigation.


This work is supported by Grants NIH 36426 to JRC, HL38650 to DEW, RR01614 and NSF DIR8700766 (to A. L. Burlingame).

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Fig. 1 A portion of the liquid secondary ion mass spectrum (LSIMS) of a RP HPLC fraction of tryptic peptides of a rabbit lung FMO preparation. Fractions typically contain more than 5 peptides. In this fraction, ten peptides were identified by LSIMS and tandem MS.

Lys-Val-His-Val-Ile-Pho-Asn-Tyr-Lys (MH+ = 1147.7)

Fig. 2 Tandem CID mass spectrum of the peptide of MH+ 1147.7 in Fig. 1. The peptide sequence can be easily read from the spectrum. It has been identified as a tryptic peptide from calreticulin, a companion protein of rabbit lung FMO.
IDENTIFICATION OF NEW ANTIBIOTIC PEPTIDES FROM *Trichoderma polysporum*

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INTRODUCTION

Trichosporin-Bs (TS-Bs) are the series of antibiotic peptides produced by *Trichoderma polysporum*. Up to now, 11 compounds have been identified by MS (FAB) and NMR, and some by FAB-MS-MS. They are blocked N-terminal peptides containing unusual amino acids such as α-aminoisobutyric acid (Aib) and isovaline (Iva), and their C-terminal is linked with phenylalanine (Pheo). However, there remain many unknown minor compounds which are extremely difficult to separate by HPLC. Their identification by FAB MS and NMR is almost impossible because of their microheterogeneity. In order to clarify their structures, linked scan and CF-FAB were performed. In this study an HPLC-separated fraction (named TS-B-I-2-1), which contained trichosporin-B-Ia (shown in Table 1 as peak f) as the main component, was examined and a new type of peptides was found.

EXPERIMENTAL

FAB MS spectra (including linked scans and CF-FAB) were obtained with CONCEPT IH (Kratos Analytical). The matrix used was glycerol : thioglycerol containing ca.1% TFA, and the assignment of quasimolecular ions was confirmed using a second matrix containing NaCl in place of TFA. Helium was used as collision gas for linked scans. For CF-FAB analysis, the mobile phase, consisting of acetonitrile : water : glycerol (50 : 45 : 5), was delivered with an LC-9A pump (Shimadzu Co.) The flow rate was 60μl/min and the analytical column used was Shim-pack SBC-ODS (2.5mmx150mm).

RESULTS AND DISCUSSION

FAB mass spectra of TS-Bs have some characteristic points:

1. The peptide bond Aib-Pro is unstable and preferentially cleaved, resulting in two acylium ions (They are called as N- and C-terminal oligopeptide ions in this paper).
2. N-terminal oligopeptides give some sequence-specific ions. The peptide bond Aib-Gin is not as stable as others and the sequence from block 7-13 can't be determined clearly.
3. Unambiguous sequence-specific ions of C-terminal oligopeptide can't be obtained.

In order to elucidate structures of minor components in TS-B-I-2-1, three steps were performed. (1) Acquiring spectra of TS-B-I-2-1 fraction. (2) Analysing the quasimolecular, N-, and C-terminal oligopeptide ions of TS-B-I-2-1 fraction by linked scan. (3) Analysing TS-B-I-2-1 fraction by CF-FAB to confirm the existence of peptides indicated by step (2).

From the results of step (1), the masses considered to be derived from peptides were examined and chosen as parent ions for linked scan analysis. The results of linked scans of quasimolecular ions gave prominent N- and C-terminal oligopeptide ions. Possible primary structures of N- and C-terminal oligopeptides were indicated from the results of linked scans of N- and C-terminal oligopeptide ions. Although a great deal of information can be obtained from linked scans of TS-B-I-2-1, the assignments are not always unambiguous. Thus, in order to gather further information, this fraction was subjected to LC separation followed by CF-FAB analysis.

Fig. 1 shows on-line detected UV chromatogram of TS-B-I-2-1. The eluate was split (30:1) for CF-FAB. The injected amount of TS-B-I-2-1 was 50 μg so totally ca. 1.6 μg peptides were introduced to the mass spectrometer. Fig. 2 shows the mass spectrum of peak e as an example. The quasimolecular ion (m/z 1937) and the fragment ions derived from N-terminal oligopeptide (m/z 1163) and C-terminal oligopeptide (m/z 744) are clearly observed. Some sequence-specific ions from m/z 1163 appear. In Table 1, peptides tentatively identified from this study are summarized. Compounds a, b, c, e, g and H have Gly at position 3. This is the first time this type of peptides has been found in *Trichoderma polysporum*.

1449
The combination of linked scans and CF-FAB is a very powerful tool in the structural determination of such a complex peptide mixture.

**Table 1** Tentatively Identified Peptidols from This Study.

<table>
<thead>
<tr>
<th>peak</th>
<th>structure</th>
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<td>(TS-B-la)</td>
<td></td>
<td></td>
</tr>
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</table>

*Lxx = Leu or Ile*

references:
1. T. Fujita et al., *J. Antibiot.*, 1988, 41, 814
CHARACTERIZATION OF BIOTIN-LABELLED PEPTIDES USING A HYBRID MASS SPECTROMETER

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Introduction

Compounds labelled with biotin exploit the strong interaction between biotin and streptavidin to provide a means of probing biochemical processes. Biotin labelling of peptides may be achieved by acylation of amine groups at the N-terminus or on amino acid side chains; there may accordingly be some uncertainty concerning the site of biotinylation in singly labelled species. We describe here a strategy for the characterization of biotin labelled peptides using a combination of trypsin hydrolysis and both conventional and tandem mass spectrometry.

We exemplify this approach using conantokin-G, a seventeen residue peptide which was the first peptide toxin reported to block the N-methyl D-aspartate (NMDA) preferring receptors with a high affinity and selectivity (1). The NMDA receptors have a unique role in brain function and their activation has been implicated in nerve death seen in stroke, epilepsy and other neuropathologies as well as in the learning and memory processes (2). The sequence is noteworthy for the presence of several carboxyglutamate (Gla) residues.

Methods

Peptides were allowed to react under lysine modifying conditions (100mM NaHCO₃, pH 9.5) with the N-hydroxysuccinyl ester of biotin. Reaction mixtures were purified by reverse phase HPLC using a 0-100% water-acetonitrile gradient with 0.1% trifluoroacetic acid. Fractions were collected and dried under vacuum. Peptides were dissolved in 200 ul ammonium bicarbonate buffer (100mM, pH 8.0) with 10% v/v n-propanol. 2 ug of TPCK treated trypsin was added and the incubations were performed at 37 C for 4 hours at which time a further 2 ug of trypsin was added and the digestion allowed to continue overnight. Products of the digestion were purified by reverse phase HPLC. Samples were analysed on a VG ZAB-SEQ Instrument of BEqQ configuration, ionization was by fast atom bombardment with a liquid matrix of either 3-nitrobenzyl alcohol or a 1:1 mixture of thioglycerol:2,2’ dithiodiethanol saturated with oxalic acid. Low energy CAD product ion scans used the qQ portion of the instrument with argon as the collision gas.

Results

Conantokin-G has two possible sites for labelling with biotin, the N-terminus and the lysine at the 15 position. The peptide was biotinylated, digested with trypsin and fractionated prior to mass spectrometric analyses. An equivalent amount of unlabelled material was treated in the same manner. Four HPLC fractions were generated and were analysed by conventional FAB mass spectrometry. MH⁺ ions were observed at m/z 1763 and 2064 for the native peptide and m/z 1763 and 746 (figure 1) for the biotin labelled peptide. The loss of the ion of m/z 2064 and the appearance of the ion of m/z 746 indicated the site of modification to be at the lysine. Low energy product ion scanning of the m/z 746 ion (figure 2) produced fragmentations consistent with the label located on the lysine. Low energy CAD of Gla-containing fragments display a propensity for losses of CO₂. In the case of the tryptic fragment 1-17 the number of residues present may be enumerated from losses of 44 from the MH⁺ ion.

References

FAB MS of Biotin Labelled Conantokin-G Tryptic Fraction 23

Gla-(Lys-Biotin)-Ser-Asn-NH₂
(Tryptic Fragment 14-17)

Low Energy CAD of Conantokin-G Tryptic Fraction 23 MH⁺, m/z 746
DETERMINATION OF THE CARBOXY TERMINUS OF THE B-CHAIN OF PORPOISE RELAXIN

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Relaxin a polypeptide hormone which is synthesized and stored in the corpus luteum during pregnancy, is responsible for the dilation of the symphysis pubis before parturition. The sequence of the A- and part of the B-chain of porpoise relaxin were obtained by Edman degradation. We used plasma desorption mass spectrometry (PDMS) to establish the MW of the A- and B-chains, to map the tryptic peptides, and (in combination with on-foil enzymatic digestion) to establish the sequence of the remaining C-terminal amino acids on the B-chain.

Molecular weights of the A- and B-chains. The relaxin treated with DTT gave MH⁺ of mass 2528.2 and 3532.6 for the A and B chains respectively (Figure 1), for a MW of the reduced A-chain of 2527.2 Da, (the MW calculated from the known aa sequence is 2527.4 Da). The MW of the B chain was determined as 3531.6 Da. When compared with the calculated mass for the known portion of the B-chain (2675.2 Da) this results in a mass deficit of 656.4 Da, corresponding to the unknown portion of the C-terminus. The MW of the relaxin can be calculated: 2527.2 + 3531.6 - 6(H) = 6052.8

Mapping of the tryptic digest. A spectrum of the tryptic digest was obtained by PDMS. The tryptic fragments are identified in structure [1]

```
[1] R|MILSEK|CCQVCCIR|K|DIAR|LC
[2] Pca-R|NDIK|ACGR|ELVR|MVVEICGSV...
```

The digest was injected on a HPLC C18 RP column. Fractions were collected, spectra obtained. In addition to the peaks corresponding to fragments of known sequence, we observed two peaks at m/z 1493.1 and 1726.1 which correspond to a fragment [11] in the C-terminus of the B-chain and that same fragment linked by a disulfide bond to the A-chain [6-11], respectively. By comparing these results with mass calculated for fragment [6] and the known portion of fragment [11], this suggest that the unknown portion of peptide [11] has a mass of:

1492.3 - 1005.3 = 487.0
1725.1 - 1237.6 = 487.5

When these results are compared with the measured mass of the B-Chain (3361.6 Da), there is an additional mass deficit of 172 Da, suggesting that there is an additional peptide [12] of mass 172 + 18 = 190 Da, that a lysine or an arginine is present at the C-terminal end of peptide [11], and the
B-chain sequence is:

\[ \text{Pca-RlTNDFIKlACGRlELVRlLWVEICGSV.. (K/R) lTA} \]  

Mass spectral analysis of purified B-chain. Reduced relaxin was injected on a RP HPLC column and fractions collected. A mass spectrum of fraction 72 gave a MH⁺ at m/z 3534.4 (corresponding to the protonated B-chain) and an additional peak at m/z 3462.8. The mass difference between these two peaks (71.6 Da) corresponds to an alanine residue. If we then subtract the mass of alanine residue (71 Da) from the mass deficit (172 Da) represented by peptide [12], this would suggest that the next residue is threonine (101 Da). Thus peptide [12] has the following sequence Thr-Ala, and the B-chain has the sequence

\[ \text{Pca-RlTNDFIKlACGRlELVRlLWVEICGSV.. (K/R) lTA} \]  

Fraction 76 shows 2 peaks, a molecular ions at m/z 3205.6 and 2877.8. The difference in mass (157.9 Da) between the peak at 3205.6 and the combined masses of the peptide [7-8-9-10-11] corresponds to an arginine residue (156.1 amu) plus a proton, suggesting arginine as the terminal residue of peptide [11]. The peak at m/z 2877.8 corresponds to the protonated molecular ion of peptide [7-8-9-10] + the known portion of [11] (1005.3 amu). If we subtract the arginine residue we get the MW of the remaining unknown residues, which is 330 amu. Therefore the sequence thus far is:

\[ \text{Pca-RlTNDFIKlACGRlELVRlLWVEICGSV (330) RlTA} \]  

"On-foll" digestion with carboxypeptidase Y. Carboxypeptidase Y was used to digest fractions 72 and 76 deposited on the sample foil. 10 minute incubations gave the best spectrum. The peaks at m/z 3533.2, 3462.6, 3361.0 3206.6 3149.2 and 2963.2 result in mass differences of 70.6, 101.6, 154.4, 57.4, and 186.0 Da, corresponding to the masses of alanine, threonine, arginine, glycine and tryptophan, respectively (figure 2). The remaining difference in mass between the mass of [7-8-9-10] + the known portion of [11] (2877.8) and the peak observed at m/z 2963.2 is 85.4 Da, which suggests that we have a serine, leading to the completed sequence of the B-chain:

\[ \text{Pca-RlTNDFIKlACGRlELVRlLWVEICGSVSWGRlTA} \]
MASS SPECTROMETRIC IDENTIFICATION OF BOVINE CHROMAFFIN GRANULE PEPTIDES

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The chromaffin cells of the adrenal medulla, more in particular the subcellular chromaffin granules, are of biological and neuropharmacological interest since they provide a model for studying the storage and secretion of neurotransmitters and hormones. The aim of our study was to confirm the presence of the known neuroregulators methionine- and leucine-enkephalin mass spectrometrically and to isolate and characterize possible unknown neuropeptides.

Peptides were extracted from the lysate of isolated bovine chromaffin granules using 0.5g C₁₈ Bond-Elut extraction columns, which were eluted with methanol:water (25, 50 and 100% methanol). Subsequently, these extracts were fractionated by reversed-phase HPLC using gradient elution with acetonitrile (Fig. 1). Methionine- and leucine-enkephalin were identified by their chromatographic retention time and by the m/z value of their protonated molecules. With regard to the unknown peptides, a number of peptides with a Mᵋ of 1745, 1445, 1332, 976 and 900 were found in the different HPLC fractions. Upon trypsinolysis, all these peptides resulted in the loss of a component with mass 545, pointing to a structural relationship and a possible common precursor. Aminopeptidase M digestion was inhibited for all the peptides examined indicating a blocked NH₂-terminus. Carboxypeptidase Y treatment of the peptide with a Mᵋ of 1745 resulted in the formation of peptides with (M + H)⁺ values at m/z 1583, 1333 and 1220, which is in agreement with the hypothesis of a common precursor. Carboxypeptidase Y digestion was also performed on the smaller peptides and revealed the amino acid sequence of 9 amino acids at the carboxyl terminus (AELDQLLHY).

The peptide with a Mᵋ of 1745 could be identified based on the daughter ions observed in the FAB/MS spectrum (Fig. 2) as the BAM (Bovine Adrenal Medulla)-1745 peptide (<EYDRVAELDQLLHY), recently described by Flanagan et al., 1990. The other peptides with a Mᵋ of 1445, 1332, 976 and 900 were characterized as smaller fragments of the BAM-1745 peptide.

In conclusion, this extraction and isolation method resulted in the mass spectral detection of methionine and leucine enkephalin and in the characterization of a series of peptides derived from the BAM-1745 peptide in bovine chromaffin granules. The BAM-1745 peptide is a processing product of chromogranin B, a quantitatively important protein in the chromaffin granules.

Figure 1
Reversed-phase HPLC chromatogram of the 100% methanol eluate of the bovine chromaffin granule lysate extracted on C18 columns. A linear gradient of 30 to 70% acetonitrile in 0.1% aqueous trifluoroacetic acid over 45 min was run at a flow rate of 0.8 ml min⁻¹. UV-detection was carried out at 214 nm. The peak numbering corresponds to the fractions collected for FAB/MS analysis. The m/z values of the most intense (M + H)⁺ signals detected in the FAB/MS spectra are indicated.

Figure 2
FAB/MS spectrum obtained for HPLC peak 6 of the 100% methanol eluate showing a protonated molecule at m/z 1746. Daughter ions observed are A₁₅ = 617; A₆ = 688; A₁₇ = 817; A₇ = 930; A₈ = 1045; A₁₀ = 1175; A₁₃ = 1537; A₁₄ = 1699; C⁶ = 733; C⁷ = 1090; D₆ = 1001. The doubly charged (M + 2H)²⁺ ion is detected at m/z 873.
**Structural Characterization of Modifications to α-Conotoxin GVIA by Mass Spectrometry**

Joseph L. Herman and Russell C. Sprensen, ICI Pharmaceuticals Group, A Business Unit of ICI Americas Inc., Wilmington, DE 19897

**Introduction:**

Conotoxins are a variety of neurotoxins found in fish-eating marine snails. These peptide compounds are relatively small in size (13-29 amino acids), and typically contain one or more intramolecular disulfide bonds. Naturally occurring α-conotoxins are of particular interest for use in the characterization of presynaptic neuronal Ca\(^{2+}\) channels. These compounds generally prevent the voltage-activated entry of calcium into the nerve terminal and irreversibly block the release of acetylcholine. α-Conotoxin GVIA is commercially available and has been the focus of recent pharmaceutical studies.

α-Conotoxin GVIA is known to consist of 27 amino acids with 3 intramolecular disulfide bridges and an amidated carboxy terminus. The structure is shown in Figure 1. Analysis of disulfide linkages is complicated by the presence of adjacent cysteines at residues 15 and 16. The secondary structure of α-conotoxin GVIA has not been previously reported. Attempts to independently verify the locations of the disulfide bonds in α-conotoxin GVIA by mass spectrometry were only partially successful.

Specific modifications are of interest in investigating the structure activity relationship (SAR) of peptides. α-Conotoxin GVIA was modified chemically by reacting native α-conotoxin GVIA with acetic anhydride. The N-terminus and lysine residues are acetylated upon exposure to acetic anhydride. For orconotoxin GVIA, which has two lysine residues, there are three possible sites for acetylation. The three possible mono-acetylated compounds, the three possible di-acetylated compounds, and the tri-acetylated compound were produced by reacting α-conotoxin GVIA with a 1:1 molar equivalent of acetic anhydride. Each of the acetylated compounds was isolated by HPLC and identified by a combination of enzymatic digests and mass spectrometry.

**Experimental:**

α-Conotoxin GVIA was purchased from Bachem. Sequencing grade tryptic and α-chymotryptic were purchased from Promega. The buffers and calcium chloride (CaCl\(_2\)) were obtained from Aldrich. HPLC grade acetonitrile and acetic anhydride were from Baker. HPLC grade trifluoroacetic acid (TFA) was purchased from Pierce. Eighteen NDI Milli-Q water was made using a Waters millipore system.

The digests were performed by dissolving 50 μg of substrate into 100 μl of a 100 mM tris, 25 mM CaCl\(_2\) solution, pH adjusted to 7.8. Enzyme was added to the solution to produce a 50:1 molar ratio of substrate to enzyme. The solution was incubated at 37°C for 16-20 hours.

Reverse phase HPLC was performed on a Zorbax RX C8 15cm x 4.6mm column. Injections of 100 μls were used. The flow rate was 1 ml/min with the following gradient: 0-2.5 min = 100% A, 0 B; 2.5-7.5 min to 50 A, 50% B; where A = 0.1% TFA in H\(_2\)O, B = 0.1% TFA in acetonitrile. The chromatographic peaks were collected, vacuum centrifuged to dryness, and redissolved in 10-μl 0.1% TFA in H\(_2\)O. HPLC conditions for separating the acetylated conotoxins were the same as for the digests except that the gradient was 0-2.5 min = 50% A, 50% B; 2.5-22.5 min to 70% A, 30% B. The mass spectral analysis was performed on a V7-70-MS mass spectrometer at 1000 resolution. Ions were produced by fast atom bombardment (FAB) using 8 kV Xenon atoms. Two μls of sample were dissolved into a matrix of 3:1 dithiothreitol:dithioerythritol (magic bullet) directly on the FAB probe prior to mass spectral analysis.

The MS/MS experiments were performed on a VG 70-SEQ tandem hybrid mass spectrometer. For these experiments, ions were produced by liquid secondary ion mass spectrometry (L-SIMS) using a 35 kV Ca\(^{+}\) ion gun. All other conditions were the same.

**Results and Discussion:**

Figure 1 shows the expected cleavages for trypsin and chymotrypsin on α-conotoxin GVIA. Two major peaks are present in the chromatograph of the tryptic/acetehyryptic digest of α-conotoxin GVIA. The mass of the (MH\(^{+}\))\(^{-}\) ion for each digest fragment found in the chromatogram was measured and is shown in Figure 1 along with their proposed structures. It is evident from the observed masses that the cysteine at position 8 is bridged to the cysteine at position 19 and that the cysteines at positions 1 and 26 are bridged to the cysteines at positions 15 and 16. (Note: Under the conditions of the digest, an unexpected cleavage at threonine 11 occurred.)

MS/MS and linked scan experiments were performed on peak 1 in order to assign the disulfide linkages to cysteines 15 and 16. However, all the ions produced by these experiments could be accounted for by fragmentation between cysteines 15 and 16. Both of these ions can also be accounted for by fragmentation of both disulfide bonds.

Eight peaks were isolated in the chromatogram of acetylated α-conotoxin GVIA. The average masses of the (MH\(^{+}\))\(^{-}\) ion were measured for each peak in the chromatogram. Peak 1 is native conotoxin, Peaks 2, 3 and 4 are mono-acetylated conotoxins, Peaks 5, 6 and 7 are di-acetylated conotoxins, and Peak 8 is the tri-acetylated conotoxin (m/z = 3036, 3080, 3122) and 3165 respectively.

The positions of the acetylations were determined by digesting the modified conotoxins with trypsin. The structure of α-Conotoxin GVIA after tryptic digestion is shown in Figure 4. All the pieces of the tryptic digest are connected by disulfide bonds except for the arginine at position 25, which is lost.

The mass of the (MH\(^{+}\))\(^{-}\) ion for the major component from the tryptic digests of Peaks 2 thru 7 are shown in Figure 4 along with their proposed structures. When the N-terminus is acetylated, a shift of 42 daltons higher than native conotoxin is observed. When the lysine at position 2 is the acetylation site, the trypptic cleavage at position 2 is blocked and the mass will be 18 daltons lower than the mass of the N-terminus acetylated compound. When the acetylation is at the lysine at position 24, the trypptic cleavage at position 24 is blocked causing the arginine at position 25 to be retained and the mass to be 156 daltons higher than the mass of the N-terminus acetylation. Therefore, each of the possible tryptic digests of α-conotoxin GVIA has a unique molecular weight relative to the tryptic digest. Peak 2 is acetylated at lysine 2, Peak 3 is acetylated at the N-terminus, Peak 4 is acetylated at lysine 24, Peak 5 is acetylated at the N-terminus and lysine 24, Peak 6 is acetylated at lysines 2 and 24, and Peak 7 is acetylated at the N-terminus and lysine 24.
**Figure 1: Primary Structure of α-Conotoxin GVIA**

Average Mass \((M+H)^{+}\) = 3038.4

B = Hydroxyproline; * = disulfide bonds; T = Tryptic cleavages; C = Chymotryptic cleavages

<table>
<thead>
<tr>
<th>Peak</th>
<th>m/z of ((M+H)^{+})</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1024.4</td>
<td>CT CK</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VACA or WCA CK CT</td>
</tr>
<tr>
<td>2</td>
<td>1450.6</td>
<td>SCHFY SBGSSCBYT</td>
</tr>
<tr>
<td>3</td>
<td>2960</td>
<td>AC SCHFY CT CARGOSCBYTNCGCA</td>
</tr>
<tr>
<td>4</td>
<td>2978</td>
<td>SCBHTK CT SBGSSCBYTNCGCA</td>
</tr>
<tr>
<td>5</td>
<td>3002</td>
<td>AC SCHFY CT CARGOSCBYTNCGCA</td>
</tr>
<tr>
<td>6</td>
<td>3134</td>
<td>AC SCHFY CT CARGOSCBYTNCGCA</td>
</tr>
<tr>
<td>7</td>
<td>3177</td>
<td>AC SCHFY CT CARGOSCBYTNCGCA</td>
</tr>
</tbody>
</table>

**Figure 2: Proposed Structures for \((M+H)^{+}\) Ions**

**Figure 3: Proposed Fragment Ions for m/z = 1024 from Peak 1 of Tryptic/Chymotryptic Digest**

**Figure 4: Structure of α-Conotoxin GVIA After Tryptic Digest**

The recent observation of multiple charging of polymeric materials has revolutionized mass spectrometry. This technique has seen application in the determination of molecular weights of many different biopolymers. However, since each individual component present in a mixture will produce a unique set of parent ions, minor impurities present in a peptide mixture can also be characterized as to molecular weight and approximate abundance. This paper describes the characterization of peptide impurities in synthetic lots of insulinotropin, a naturally occurring insulin secretagogue.

Peptide lots were dissolved in a 50/50 mixture of acetonitrile/1% formic acid such that a peptide concentration of approximately 1 ng/μL was obtained. These samples were introduced directly into the mass spectrometer without chromatographic separation.

All analyses were performed on a SCIEX API III mass spectrometer fitted with an ionspray probe. Samples were infused at 10 μL/min into the API ion source using a syringe pump. The mass spectrometer was operated in the positive ion mode and spectra were acquired from m/z 600 to 1600 daltons. Molecular weights of detected peptides were calculated from manually selected ions using a software routine supplied with the instrument.

Ionspray of peptides produces multiply charged ions in a pattern that is dependent in part on the number of basic amino acids in the molecule. Insulinotropin produces three ions in its ionspray spectrum as indicated in Figure 1. A simple calculation using the masses of these three ions produces a molecular weight of 3355 daltons. This calculated value is in excellent agreement with the theoretical value of 3355.7 daltons.

If more than one peptide is present in the sample, multiply charged ions for each component of the mixture are observed and their molecular weights can also be calculated. Figure 2 shows the ionspray spectrum of an insulinotropin bulk containing several minor components. Nine different peptides were identified in eight different bulk samples (Table 1). Seven impurities appear to represent the loss or gain of a single amino acid residue from insulinotropin.

Data on the impurities was obtained by manual manipulation of the mixture spectra. Software based on the deconvolution algorithm described by Mann et al. could be used to automate this process.

Figure 1. Ionspray API spectrum of insulinotropin

Figure 2. Ionspray API spectrum of a mixture of insulinotropin and impurities.

TABLE 1. Insulinotropin impurities by Ionspray API.

<table>
<thead>
<tr>
<th>Peptide M.W.</th>
<th>Loss/Oxin from FT</th>
<th>Change</th>
<th>Theoretical M.W.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3412.1</td>
<td>+57.1</td>
<td>+GLY</td>
<td>3412.8</td>
</tr>
<tr>
<td>3217.7</td>
<td>-136.1</td>
<td>-HIS</td>
<td>3218.8</td>
</tr>
<tr>
<td>3254.6</td>
<td>-180.1</td>
<td>-THR</td>
<td>3254.7</td>
</tr>
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<td>3287.6</td>
<td>-87.9</td>
<td>-SER</td>
<td>3288.7</td>
</tr>
<tr>
<td>3297.8</td>
<td>-58.2</td>
<td>-GLY</td>
<td>3298.7</td>
</tr>
<tr>
<td>3422.0</td>
<td>+158.3</td>
<td>+HIS</td>
<td>3482.9</td>
</tr>
<tr>
<td>3197.6</td>
<td>-185.7</td>
<td>-THR; -GLY</td>
<td>3197.5</td>
</tr>
<tr>
<td>3240.0</td>
<td>-115.7</td>
<td>-ASP</td>
<td>3240.6</td>
</tr>
<tr>
<td>2309.3</td>
<td>-1046.5</td>
<td>?</td>
<td>7</td>
</tr>
</tbody>
</table>

Calculated M.W. = 3355.2 ±0.1 daltons
Theoretical M.W. = 3355.7 daltons
THE IDENTIFICATION OF SYNTHETIC PEPTIDE PROCESS IMPURITIES BY ON-LINE CONTINUOUS-FLOW FAB ANALYSIS OF CARBOXYPEPTIDASE DIGESTIONS.

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The investigation of peptides as therapeutic agents has spurred the use of mass spectrometry as a sequence determination tool. This is especially true in the case of N-terminally blocked or structurally modified peptides in which traditional techniques such as automated Edman degradation fail.

Tandem mass spectral techniques have had limited success when attempted to sequence peptides of unknown composition, as is the case with process impurities. A more selective mass spectral method is provided by following the course of an exopeptidase digestion with an on-line device such as continuous-flow FAB (CF-FAB).

A solid-state peptide synthesis of U-86364E, intended to give the N-terminal FMOC protected peptide, was characterized by low recovery from the resin and the concomitant appearance of two major and two minor impurities.

LYS-ARG-ALA-LYS-ALA-LYS-THR-THR-LYS-LYS-ARG
U-86364E

Initially it was suspected that the four impurities were the result of incomplete deprotection during recovery from the resin. The four protecting groups used were as follows:

- FMOC
- Tosyl
- ChloroZ
- Bz

The M+1 ions obtained by FAB for the four impurities were:

<table>
<thead>
<tr>
<th>Compound</th>
<th>M+1 Ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1627.7</td>
</tr>
<tr>
<td>B</td>
<td>1692.2</td>
</tr>
<tr>
<td>C</td>
<td>1662.7</td>
</tr>
<tr>
<td>D</td>
<td>1816.5</td>
</tr>
</tbody>
</table>

Compounds A and B corresponded to U-86364E+FMOC+Bz and U-86364E+FMOC+Tos, respectively. The molecular weights of the remaining two impurities were justified by invoking a decomposition of the ChloroZ protecting group to a Bz-Cl:

\[
\text{ChloroZ} \rightarrow \text{Bz-Cl}
\]

Compound C therefore corresponded to U-86364E+FMOC+Bz-Cl and compound D to U-86364E+FMOC+Tos+Bz-Cl.

The supposition at this point was that the impurities were incomplete deprotection species, however, repeated HF treatment did not remove the protecting groups. Determination of the location of the protecting groups was attempted using tandem mass spectrometry but was not successful; daughter ion spectra were dominated by an ion corresponding to the base peptide U-86364E.

An enzymatic digestion using Carboxypeptidase P was allowed to proceed over the course of a number of hours and monitored by CF-FAB. The results for compound A are presented in the following figure:
In each case it was possible to observe cleavage of the peptide down to an ion corresponding to FMOC, lysine, and the corresponding protecting group. These data reveal that the impurities were not the result of incomplete deprotection but the result of migration of the protecting groups to the N-terminal end of the peptide.

What remained to be confirmed was whether these protecting groups had migrated to the N-terminal FMOC. 13C-NMR DEPT experiments indicated the impurities consisted of the appropriate aromatic substitutions. Two-dimensional NMR experiments are in progress to determine the exact site of substitution.

Experimental Procedures:
Mass spectral data were collected and processed on a Finnigan TSQ-70 triple stage quadrupole mass spectrometer. FAB was acquired using Xenon atoms generated by an Ion Tech saddle field gun operating at 8KV in static mode and 5 KV in continuous-flow (CF-FAB) mode. CF-FAB was performed in 5% glycerol/water and flowed to the source at 10μl/minute. Excess matrix was absorbed onto a circular piece of filter paper which had been fitted around the probe tip. The probe, manifold, and source were kept at a temperature of 30°C, 50°C, and 55°C, respectively.

The peptides were constituted in 150μl of pH Milli-Q filtered water, at a concentration of 2 nmole/μl. Carboxypeptidase P was added such that the substrate:enzyme ratio was 100:1. After the designated time interval 23μl of digest were removed and quenched with 2μl of 20% TFA. Ten microliters of this aliquot were immediately loaded into the CF-FAB injection port and mass spectral data collected scanning from 500 amu to molecular weight plus 50 amu. A second 10 μl aliquot was injected immediately after and used to scan the low mass range from 50 amu to 500 amu.

References:
Structural characterization of cysteine proteinases isolated from lobster gastric juice using LC-MS enzymatic mapping and on-line LC-MS-MS

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Cysteine proteinases such as papain are quite distinct from other endopeptidases as their enzymatic activity depends on the presence of a free thiol group of a cysteine residue, located within the catalytic site [1]. Recently a new cysteine proteinase, which accounts for 80% of the proteolytic activity in the lumen of the lobster hepatopancreas, was purified in this laboratory [2]. Further work was conducted to clone DNA sequences that encode this enzyme using oligonucleotide probes synthesized using information from the N-terminal amino acid sequence previously reported [2]. This latter work yielded three distinct cysteine proteinase clones having approximately 210 amino acids and average molecular weights of 23066, 23270 and 23377 Da.

Mass spectral analyses using Ionspray were conducted to characterize the proteinases found in the gastric juice of the lobster, and to relate these to the amino acid sequences encoded in the cDNA clones. These analyses were also undertaken to determine sites of post-translational modification, in particular disulfide bridge location. Purified lobster cysteine proteinase extract was first denatured to minimize the effect of protein folding. A portion of this preparation was then alkylated using iodoacetamide both with and without prior disulfide bond reduction. Figure 1 shows the Ionspray mass spectra of the denatured proteins, and indicates two coherent series of multiply-charged ions corresponding to molecular weights of 23378 and 23085 Da respectively. These masses were in close agreement with those predicted from two of the proteinase clones. Alkylation of free thiol groups resulted in a mass shift of approximately 57 Da for both proteinases, indicating the presence of only one free cysteine in each case. Upon reduction/alkylation the multiply-charged ion envelope is shifted toward lower m/z ratios, consistent with the protein unfolding and a higher number of available protonation sites. Also, the masses of the two proteinases were shifted by 590 and 567 Da respectively, implying a total of 10 cysteine residues in each case. The higher number of alkylation sites could be explained by reaction of other amino acids such as histidine.

Primary structure characterization of these two proteinases was investigated by analyzing tryptic digests of denatured and alkylated proteins using Ionspray LC-MS. An example of such analyses is presented in Figure 2a, which illustrates the reconstructed ion chromatogram (m/z 600-1400 Da) of the tryptic digest of reduced/alkylated proteinases. Alkylated cysteine peptides were rapidly identified by both their shift toward lower retention times and their incremental shift in mass with respect to their underivatized counterparts. Finally, amino acid sequence assignment was confirmed using LC-MS-MS analyses of selected tryptic peptides. Figure 2b shows the fragment ion mass spectrum of the doubly-charged peptide T79_94 of the major proteinase (peak 1 on Figure 1). This on-line LC-MS-MS shows almost complete peptide sequencing using less than 100 pmols of total protein digest.

*NRCC# 32978
Figure 1: Ionspray mass spectrum of mixed cysteine proteinases from lobster digestive gland. Infusion of 10 µg (approx 200-300 pmole of proteins) dissolved in 10% glacial acetic acid.

Figure 2: LC-MS and LC-MS-MS analysis of tryptic digest of reduced and alkylated cysteine proteinases from lobster digestive gland: (A) Reconstructed ion chromatogram (600-1400 Da). (B) Fragment ion spectrum of precursor ions of m/z 880, [MH]^{2+}_{2} of T_{79,94}. Number in parenthesis correspond to tryptic peptide of either the major (1) or minor (2) cysteine proteinase. Asterisks indicate peptides containing alkylated cysteine residues. Inj. of 5 µg of total protein digest on a 25 x 0.1 cm C18 column, 10-50% acetonitrile (0.1% TFA) in 20 min, 50 µL/min with no split.

INTRODUCTION

High resolution of high-energy, high-mass ions in a Fourier Transform Ion Cyclotron Resonance (FT-ICR) Mass Spectrometer has proven to be difficult or impossible to obtain. This may be due to varying or unknown parameters or to unforeseen nonlinearly varying inputs. Practical limits may be made on design variables as well as generic computerized models. Simulation studies show the possibilities of effectively resolving high-energy, high-mass ions in the mass range beyond that which is obtainable in the present day FT-ICR mass spectrometer. Recent development in FT-ICR mass spectrometry may pave the way to more promising design and operation parameters necessary for high-energy, high-mass resolution. Such analytical instruments would promise high performance superiority over present day methods. The possibility of defining such parameters is considered. The study will focus on FT-ICR ion mass resolution design equations whose variables will be tested for optimum performance.

EXPERIMENTAL

All simulations were done using IBM computer. The computer simulations assume totally homogeneous electric and magnetic fields gradient, ideal cell dimensions and design, and non-reactive hard-sphere ions. The computer program is based on analytical solution of the equation of motion for the ions accelerated by an applied rf electric field. Only ions in a X-Y plane are addressed by the program to allow specific studies of radial motion to be performed. Variables of the design equations were tested and the search for optimum ranges of variable parameters was conducted.

RESULTS AND DISCUSSION

The final radius distribution was plotted versus the frequency sweep rate figure 1. The plot shows that at varying sweep rates ions will obtain different degrees of coherence. The level of coherence seems to vary in an oscillating manner. That is the same level of coherence is obtained at different sweep rates. Thus different degrees of resolution will be obtained depending on the sweep rate used for excitation and from the data it is possible to obtain the same results at different sweep rates.

The effect of voltage on ions coherence was tested for consideration as one of the essential forces required to coerce randomly distributed ions into coherence. Four different sweep rates were tested varying the excitation voltages. The number of cycles to obtain the same level of coherence were normalized and plotted versus the excitation voltage change. The behavior of the irradiltated ions under electromagnetic effect is described in plots of their trajectories as shown in figures 2. Attempts to ensemble ions at low voltage was not very successful. At such insufficient voltage, ions lacked sufficient energy to overcome the diffusive and repulsive forces that dominate similar-charge ions. Thus it would take a larger number of cycles to obtain any degree of
coherence no matter what the sweep rate was. At high voltage, the ions acquired sufficient energy to achieve phase advancement, ensemble, and radially accelerate. It was observed that ions with ideal sweep rates would obtain coherence at fewer cycles of oscillation at higher excitation voltages.

CONCLUSION

The accompanying plots and illustrations show what should happen theoretically and what happens in the computer simulation. The plots show two ions which have different phase angles due to the difference in the times of their formation. In order to achieve a good signal, the ions must be in phase with each other and the applied electrical field before they reach maximum excitation. The plots of voltage vs. frequency show that with a high voltage the ions can be driven into phase quickly, but if the voltage is too high, the ions' radii exceed the limitations of the instrument's cell. The steps in which the frequency is swept also determine whether or not the ions go into phase. Smaller increments seem to drive the ions into phase while larger increments drive them further out of phase than is caused by their times of formation.
INTRODUCTION

The 69-kDa outer membrane protein (OMP) of *B. pertussis* is considered to be a potentially useful vaccine component in acellular vaccines and therefore has been subjected to further characterization. Automated Edman degradation through 20 cycles showed that the N-terminal sequence corresponds to that predicted from the DNA sequence. C-terminal analysis by carboxypeptidase Y digestion gave ambiguous results due to co-elution of Ser and Asn. These data were not sufficient to assign a specific C-terminus but did indicate that the putative termination site at residue 677 which corresponds to a molecular mass of 69 kDa was incorrect. Instead, the data were compatible with sequences found between residues 597-599 and 597-600. In addition to the 69-kDa species, certain protein preparations showed a doublet on SDS-PAGE with a second band at ca. 67 kDa. With time, it appeared that the 69-kDa species could be completely converted to 67 kDa, and therefore it was assumed that the conversion was due to adventitious proteolysis. Alternatively, sequence analysis was identical to that from the 69-kDa species, implying C-terminal cleavage to yield the 67-kDa product.

HIV reverse transcriptase (RT) p51, a part of the heterodimer p51/p66, is produced by cleavage of p66 by HIV protease. In order to rapidly determine the exact cleavage site, the protein was subjected to various forms of mass analysis coupled with chemical and enzymatic degradation.

PROBLEMS

a) Determine/verify N- and C-termini of natural and recombinant 69 kDa pertussis outer membrane protein relative to the DNA-derived sequence by measuring molecular masses. Also, determine/verify the N- and C-termini of the natural 67 kDa form that appears to result from adventitious proteolysis. b) Establish location of cleavage site in HIV reverse transcriptase p56 that yields p51 of the heterodimer.

DISCUSSION

Pertussis Outer Membrane Protein. Analysis of each protein by electrospray MS yielded spectra for both the 69-kDa OMP and the 67-kDa protein. Close examination of each spectrum revealed that the peaks were wider than is typical for ESMS spectra suggesting the presence of components contributing to peak broadening. Other experiments (e.g., FABMS of limited tryptic digests) suggested the cause to be cations (Na+, K+) and, for the 67-kDa protein, a ragged C-terminus (see below). Careful evaluation of the peaks resulted in reasonably accurate mass measurement. In separate experiments on different protein preparations, molecular weights of 60,297±16 and 60,291±8 were measured for the 69-kDa protein. A molecular weight of 58,132±8 was measured for the 67-kDa protein. These data are consistent with sequences of Asp-Asn and Asp-Ala for the 69-kDa and 67-kDa species, respectively.

To confirm these conclusions, the two proteins were subjected to limited trypsin hydrolysis (15 min; E:S 1:1000) and size exclusion HPLC. The peptide fraction from the hydrolysis of the 69-kDa OMP gave a readable sequence of 27 amino acids by automated Edman analysis corresponding to the expected trypsin fragment Glu-Ala-Ser-Ala-Ala-Ala-Asn. ESMS analysis of the tryptic peptides was in agreement with the Edman analysis. The peptide fraction from the hydrolysis of the 67-kDa degradation product was also analyzed by automated Edman analysis yielding a readable sequence of seven residues: Glu-Leu-Ser-Ala-Ala-Ala-Asn. However, ESMS analysis showed a mixture of peptides of molecular masses of 489, 560, and 674 Da corresponding to Glu-Ala, Glu-Ala, Glu-Ala-Asn, respectively, indicating a ragged C-terminus for the 67-kDa species.

Molecular mass measurements of the intact 69-kDa OMP and the peptides released by limited trypsin digestion are consistent with Asp-Asn from the DNA-derived sequence of Charles et al. (PNAS, USA, 1989, 86, 3554) corrected for sequencing errors. These errors (Leu→Pro and Pro→Ser), which were detected by amino acid analysis of internal tryptic peptides (data not shown) and correspond to single base pair replacements, produce mass shifts of -16 and -10 Da, respectively. Note that our data are equally consistent with the uncorrected DNA-derived sequence (M_calc = 60,308). Molecular mass measurements of the recombinant 69-kDa OMP and the peptides released by limited trypsin digestion are consistent with the sequence Met-Ser-Glu-Ala-Ser-Glu-Ala-Asn. Molecular mass measurements of the intact 67-kDa OMP are consistent with the sequence Asp-Ala, and the peptides released by limited trypsin digestion are consistent with the presence of sequences Asp-Ala, Asp-Ala, Asp-Ala, Asp-Ala-Asn, respectively. The peaks corresponding to protein molecular ions for the ragged C-terminus could not be resolved due to the presence of cations (Na+, K+) or other adducts.

HIV Reverse Transcriptase p51. In a similar fashion, the p51/p66 heterodimer of HIV reverse transcriptase (RT) was analyzed by ESMS. The data though weak showed two major series corresponding to masses of 51,298±5 (M_calc = 51,302) and 64,494±10 (M_calc = 64,432). Analysis by LDMS showed M_calc = 51,451 and 64,451. The LDMS data were acquired in nominal mass mode, without internal calibration references, and therefore the masses are only approximate.) In order to corroborate the cleavage site which distinguishes p51 from p66 (believed to be near residue 432), p51 was isolated and subjected to oxidative cleavage at tryptophan followed by FABMS. A peak was observed at m/z 1624.6 which reasonably corresponds only to Tyr-Phe. To verify the assignment of the m/z 1624.6 peak, a Lys-C digest was performed on the putative C-terminal peptide. FABMS analysis of this digest gave an expected peak at m/z 963 which corresponds to Glu-Phe which was further confirmed by one cycle of manual Edman degradation.
CONCLUSION: These examples illustrate some of the limitations of both MS and automated Edman analysis. In the ESMS data of the "67 kDa" OMP, the presence of a ragged C-terminus was not observable in the presence of intractable cationic contamination. Even under ideal conditions on a pure sample, the presence of a ragged C-terminus with differences of +1 and +2 amino acids would have been barely perceptible for a protein of this mass; the difference in mass for an additional Ala (71 Da) divided by the charge state (e.g., 50+) gives a difference in m/z of only 1.4, which is approaching the theoretical peak width for these proteins. In automated Edman analysis, quantitation may not be sufficiently reliable to establish the presence (or absence) of mixtures. Such analyses should be coupled with analysis of the peptide fraction by RP-HPLC with UV detection for better quantitation (temporal separation) and MS for greater selectivity (mass separation). HPLC/MS with simultaneous UV detection provides a powerful solution to both problems.

Figure 1. ESMS analysis of ca. 300 pmol of B. pertussis 67-kDa OMP. The sample (ca. 20 pmol/μL in 3:7 MeOH/H₂O containing 2% acetic acid) was introduced into the mass spectrometer at 2-3 μL/min and data collected for ca. 6 min.

Figure 2. ESMS analysis of ca. 150 pmol of the limited trypsin digest (15 min, E:S = 1:1000) of B. pertussis 67-kDa OMP. The sample (ca. 20 pmol/μL in 3:7 MeOH/H₂O containing 2% acetic acid) was introduced into the mass spectrometer at 2-3 μL/min and data collected for ca. 3 min.
HIV-gp120 is the surface receptor of the AIDS virus. This glycoprotein is believed to mediate viral attachment to host cells through the CD4 receptor. Due to the critical role that gp120 plays in the life cycle of HIV, it is of possible interest as a therapeutic target and as a vaccine candidate. The glycoprotein expressed in Drosophila contains 454 amino acids and 24 possible N-linked glycosylation sites, and is known to be heavily glycosylated. The carbohydrate moieties of gp120 may have important roles in the activity of the glycoprotein since deglycosylation results in diminished binding activity of gp120 to CD4\(^1\). The 44 amino acid of gp120 that is believed to be responsible for binding to CD4\(^1\) contains one possible glycosylation site that was found to be filled. Additionally, the V\(_\alpha\) loop, which is thought to be the portion of gp120 that generates neutralizing antibodies, contains one, and is bounded by two possible glycosylation sites, all of which have been found to contain carbohydrate. The objective of the current study was to develop mass spectrometric and liquid chromatographic methods to confirm the expected sequence of recombinant gp120, determine which of the 24 possible glycosylation sites are utilized, and ascertain the structural class, branching, and molecular heterogeneity of the carbohydrate units at each attachment site.

Peptide and carbohydrate mapping of recombinant gp120 was accomplished by LC-ESMS. Reduced and carboxymethylated gp120 was subjected to enzymatic digestion prior to and after release of the carbohydrate moieties with PNGase F (Figure 1). Three different enzymatic cleavage schemes were employed so that glycopeptides that contain only one glycosylation site could be produced for a majority of the 24 possible attachment sites. These include a tryptic digestion, a tryptic digest followed by endoproteinase Glu-C, and tryptic digestion followed by asparaginylendopeptidase cleavage. The molecular weights of the resulting peptides from each enzymatic digestion were observed by LC-ESMS and fitted to the deduced sequence of the glycoprotein. Some glycopeptides were also observed by LC-ESMS and yield information concerning structural class and microheterogeneity of the carbohydrate units. PNGase F release of the oligosaccharide moiety converts Asn to Asp, which aids in the determination of attachment sites since Asp weighs one more dalton than Asn. Possible glycopeptide containing fractions are identified by comparing UV and ESMS of digested RCM-gp120 and PNGase treated RCM-gp120.

Putative glycopeptide containing fractions were then subjected to direct mass spectral analysis followed by PNGase treatment. The former glycosylation site peptide was then identified by MS analysis. Portions of the PNGase reaction mixtures were retained for high performance anion exchange chromatography to obtain a high resolution carbohydrate fingerprint at each site, and the remaining material was permethylated with DMSO/NaOH/Mel. The permethylated oligosaccharides can be analyzed by FAB or ESMS and methylation analysis. This strategy enables a great deal of information regarding structural class, microheterogeneity, and branching to be gathered very quickly.
Using this strategy, more than 95% of the polypeptide portion of gp120 has been mapped. Of the 24 possible glycosylation sites, 18 have found to be filled and 3 partially occupied. We have found that recombinant gp120 from Drosophila contains primarily oligomannose type carbohydrate structures (Man$_n$-Man$_8$). Different attachment sites tend to exhibit varying amounts of carbohydrate processing. The extent of glycosylation observed in the mapping studies is consistent with the mass of 83,000 Da obtained by laser desorption time-of-flight mass spectrometry (B. Chait personal communication). The average mass of the polypeptide core of gp120 is 50,777 Da indicating that 36% of the glycoprotein is carbohydrate.

RecA (recombinase A) is a 38 kilodalton enzyme which is essential for genetic recombination and repair in E. coli. The sequence of recA has been deduced from a gene sequence and partially verified by amino acid analysis. The protein binds to DNA, ATP and ADP. A three-dimensional structure of recA was recently proposed based on x-ray crystallography data obtained for recA bound to ADP. Traditionally chemical modification of proteins has been used as a tool to study their three-dimensional structure and locate binding sites. Chemical modification studies of recA have indicated that in the presence of ATP two of the three cysteines present in the recA sequence are protected from reaction with a sulphydryl modifying agent. In the absence of ATP all three cysteines react with the modifier, but as ionic strength increases one cysteine becomes protected. The position of this protected residue could not be identified using amino acid analysis. We are using mass spectrometric techniques to determine the extent and location of chemical modification of recA protein under various reaction conditions in an effort to study the three-dimensional structure of recA protein in solution and locate DNA, ATP and ADP binding sites.

When analyzed by direct injection using a Finnigan TSQ 70 quadrupole fitted with a Finnigan MAT electrospray ion source, a 500 ng injection in 60% methanol/5% acetic acid in water of recA yielded a series of multiply-charged ions. The deconvolution of this series of peaks resulted in an average molecular weight of 37,852. This represents an error of 0.03% from the predicted value of 37,842. In addition, analyses of the recA tryptic digest by standard FAB MS, continuous-flow FAB MS and LC/electrospray MS confirmed 92% of the predicted sequence for recA protein.

RecA protein was treated with N-ethylmaleimide (NEM) in a 15-fold molar excess and incubated for ninety minutes. NEM reacts with sulphydryl groups and increases the mass of cysteine residues by 125 u. RecA contains three cysteines at positions 90, 116 and 129. The modified recA protein was analyzed by electrospray MS. The resulting envelope of multiply-charged ions was deconvolved to yield an average molecular weight distribution which indicated two to three residues had been modified. An expansion of the deconvoluted spectrum is shown in Figure 1. The peak at 37,843 daltons corresponds to unmodified recA with a predicted molecular weight of 37,842. The peak at 37,970 daltons corresponds to recA modified by one NEM group with a predicted molecular weight of 37,967; that at 38,097 daltons to recA modified by two NEM groups with a predicted molecular weight of 38,092; and that at 38,201 daltons to a small amount of recA modified by three NEM groups with a predicted molecular weight of 38,217 daltons. An expansion of the deconvoluted spectrum for recA which was not treated with NEM is shown in Figure 2 for comparison.

To locate the sites of NEM-modification within the recA sequence, a tryptic digest of NEM-modified recA was analyzed by standard FAB MS. The three cysteines of recA are contained in two tryptic peptides: (89-105) at m/z 1907.1 and (107-134) at m/z 2974.3. These peptides are clearly seen in the spectra for the tryptic digest of unmodified recA. The tryptic digest of modified recA yields a peak at m/z 2032.2, which represents (89-105) modified by one NEM group, a peak at m/z 3100.7, which represents (107-134) modified by one NEM group and a peak at m/z 3224.3 which represents (107-134) modified by two NEM groups. The peak at m/z 2032.2 indicated cys 90 has been modified. The peak at m/z 3224.3 shows both cys 116 and cys 129 have been modified. However, the single site of modification for the peptide at m/z 3100.7 cannot be differentiated between cys 116 and cys 128. The m/z values for peptides which do not contain cysteine were unchanged by treatment with NEM.

Our findings show that electrospray can be used to monitor the extent to which recA reacts with NEM under specific reaction conditions. Tryptic FAB-mapping can be used to locate a modification of cys
90. However, this technique alone cannot differentiate between modification of cysteine 116 and 129. We will employ tandem mass spectrometric analysis of the peptide at m/z 3100.7 for this purpose. Further studies are underway which will utilize mass spectrometry to study the chemical modification of cysteine and other recA residues under a variety of reaction conditions and in the presence of coenzymes and substrates such as DNA, ATP and ADP.

References


Acknowledgements

The authors gratefully acknowledge Robert Reiser of Dupont for his gift of the capillary C_{18} column used in the LC-ESI MS analysis, Piero Blanco of the University of Texas Medical School, Houston for generously supplying purified recA protein and the NIH for support of this research.

Figure 1. Deconvoluted electrospray spectra of recA protein treated with NEM.

Figure 2. Deconvoluted electrospray spectra of native recA protein.
Fragmentation of Proline Containing Peptides

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Proline has been reported to influence the collisionally induced fragmentation of peptides in characteristic ways. Biemann has pointed out its inability to form d series ions (1), and Hunt reported that peptides containing proline produced decomposition products by double cleavage of the peptide chain (2). Williams has suggested that formation of pairs of y and y' ions is characteristic of proline residues (3). While sequencing S. aureus V8 protease, we have acquired more than a score of peptides with proline in every third position (4).

This set of peptides has allowed us to evaluate the effect of proline on fragmentation initiated by different kinds of ion activation. Three types of fragmentation have been studied: high energy collision induced decomposition (CID), reaction induced decomposition (RID), and collisional activation of multiply charged ions produced by an electrospray ionization source (ESD). For CID and RID, the peptide samples were dissolved in 0.1% trifluoroacetic acid and added to either thioglycerol or 3-nitrobenzyl alcohol before being introduced into the FAB source. CID spectra were obtained using all four sectors (EBEB) of a JEOL HX110/HX110 high performance mass spectrometer with resolution set at 1000. Helium was used as the collision gas between the two mass spectrometers at a pressure sufficient to attenuate the precursor ion beam by 80%. The accelerating voltage was 10 kV, and the collision cell was floated to 4 kV. For the RID experiments, ammonia was used as the reaction gas, and the collision cell was maintained at 10 kV - Vlab where Vlab = Elab/e and Elab = beam energy. Product ion spectra were obtained for beam energies ranging from 1 to 60 eV, by scanning the magnetic field in MS-2. The ESD was done with a Vestec electrospray ionization source coupled to a Hewlett Packard 5988A quadrupole mass spectrometer. Fragmentation was achieved by changing the repeller voltage.

All three kinds of fragmentation are illustrated in Figure 1 for a synthetic peptide, DNPNNPDPNNPNPD. The high-energy CID fragmentation is quite complex when compared to the low energy reaction induced fragmentation. Preferential reaction and subsequent fragmentation occurs at peptide bonds where proline contributes the amine group. The electrospray fragmentation shows most of the y series ions this peptide could generate, simplifying the interpretation relative to the CID spectrum also. In all cases studied, the RID spectrum shows proline amide bond cleavage almost exclusively. This work demonstrates progress in controlled fragmentation leading, we think, to improved mass spectrometric sequencing protocols for peptides and proteins.

This work was supported by grants from the National Science Foundation and the National Institutes of Health.

FAB-MS ANALYSIS OF A MASTOPARAN B ISOLATED FROM THE HORNET (VESPA BASALIS) VENOM WITH B/E LINKED SCANNING

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INTRODUCTION

Vespa Basalis is one of the most dangerous hornets found in Taiwan. The incidence of sting death caused by this hornet is high and is probably due to its aggressiveness and highly toxic venom. Freshly collected venom was fractionalized into 13 fractions on a Fractogel TSK HW 50 gel filtration column. Fractions 3 and 6 were further purified from the venom after cation-exchange chromatography on CM-Trisacryl M. Fraction 3 showed the highest lethality and was estimated to have approx. 32,000 daltons molecular weight by gel electrophoresis and amino acid analysis (1). Fraction 6 was a tetradecapeptide, designated as mastoparan B, possessed the potent hemolytic activity and synergized with the lethal fraction 3. It was suggested that mastoparan B might possibly involve the lethal effect of Vespa Basalis venom. The sequence of mastoparan B from position 11 to 14 was difficult to ascertain by Edman degradation method. The aim of this study is to determine the amino acid sequence of the mastoparan B using FAB-MS with B/E linked scanning.

EXPERIMENTAL

A 1-μL aliquot of the peptide solution (approx. 5-μg peptide dissolved in 5-μg water) was mixed with 1-μL of thioglycerol matrix on the FAB probe tip. The mass spectrometric analysis were carried out with a two-sector magnetic deflection mass spectrometer (JEOL HX-110, EB geometry) operated either in the normal scan or in the linked scan mode. Ionization was achieved by bombardment with a 6-KeV Xe° beam and the accelerating voltage of the mass spectrometer was operated at 10-KeV. (CsI)nCs+ cluster ions are used for mass calibration. High resolution measurements for ions in the normal FAB spectrum were made at the resolving power of 10,000, otherwise the resolving power was set at 3,000. The normal FAB spectrum was collected in 10 sec. In the linked scan mode, the collision-induced decomposition (CID) spectrum was obtained by admitting the Helium into the collision cell (first field-free region) at the pressure (approx. 10^-4 ~ 10^-6 torr) that the precursor ion was attenuated to 50% of its original abundance. The linked scan at constant B/E ratio was generated by the JEOL JMA-DA5000 data system. The single scan raw data profile was recorded in 1 min.

RESULTS AND DISCUSSION

Peaks of (M+H)+ and (M-H)- with m/z = 1612 and 1610 are observed from the normal positive and negative FAB spectrum respectively. The protonated molecular ion of the peptide was further ascertained to be m/z = 1612.01 from the
high resolution measurement and the formation of \( (M+Na)^+ = m/z = 1634 \) and \( (M+K)^+ = m/z = 1650 \) by adding a small amount of 0.1 M NaNO₃ and KNO₃ solution to the sample. It is found out that the carboxylic acid in the C-terminus is converted to an amide group. The characteristics of the normal FAB mass spectrum that the lack of continuous series of ions of one type (the various ion types proposed by Roepstorff and Fohlman (2)) and the difficulty of interpreting the mass spectrum in the low-mass region due to the matrix interferences are evident. The attributes of the CID mass spectra of peptide exhibiting extensive series of the same ion type and reduced matrix interference effects via the appropriate selection of the precursor ion are exploited to overcome the aforementioned shortcomings. The illustrative example is shown in Figure 1, which represents the CID spectrum of \( (M+H)^+ = m/z = 1612 \) protonated molecular ion. The y-ions are of \( m/z = 1499, 1371, *, 1130, 1043, *, 831, 744 \), which corresponds to the partial sequence of Leu-Lys-Leu-Lys-Ser-Ile-Val-Ser. The position labelled with * indicates a missing y-ion and the corresponding amino acid is determined using the dipeptide residue masses. The dominant b-ions are of \( m/z = 1482, 1383, 1255, 1127, 1056, 869, 782, 683, 570, 352, 242 \), which corresponds to the complete sequence of NH₂-Leu-Lys-Leu-Lys-Ser-Ile-Val-Ser-Trp-Ala-Lys-Lys-Val-Leu-NH₂. It is noted that certain b-ions with the measured \( m/z \) from 869 down to 242 are all one mass unit smaller than the expected \( m/z \). Similar, but less, amount of information are also obtainable from the CID spectrum of \( m/z = 1028 \). In both cases, the differentiation of Gln/Lys and Leu/Ile are clarified with the partial information from the amino acid analysis and the Edman degradation method.

REFERENCES


Figure 1. CID daughter spectrum of the \( (M+H)^+ \) of Mastoparan B.
Sequence-Informative fragmentation in \(^{252}\text{Cf}\)-Plasma Desorption Mass Spectrometry

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Plasma desorption mass spectrometry (PDMS) has been reported to yield sequence-informative fragmentation several times now.\(^1\) Fragmentation in oligopeptides beyond the scope of collisionally activated dissociation (CAD) using sector instruments was first observed for ions up to 4.6 kDa, but the upper limit has been extended to 8.9 kDa.

Fragmentation of ions of this size is far beyond the scope of CAD of macromolecular ions using sector instruments. On the one hand, it raises questions about the upper limits of sequencing by PDMS. On the other hand, it poses queries about the ionization process and the physics involved. The coupling among the many oscillators present in the molecule would seem to be low in the involved time-frame and the energy pumped into the molecule by the ionization/desorption process would seem to remain quite localized during the fragmentation processes which take place within nanoseconds at most.

Another aspect of the fragmentation is the pattern of it. What types of fragment ions are usually observed? In our experience we often observe \(a\)-ions, \(d\)-ions, \(x\)-ions, \(w\)-ions and \(z\)-ions. We seldomly observe \(b\)-ions, \(c\)-ions, \(y\)-ions and \(y\)-ions. The ion types we observe indicate a high internal energy content of the ions. The spectra of some peptides only show \(a\)-ions whilst that of other peptides show \(a\), \(d\), \(x\), \(w\) and \(z\)-ions. This is undoubtedly a consequence of the primary structure. It would be interesting to know the influence of the secondary and/or tertiary structure on the fragmentation pattern. When we look to the fragmentation pattern of the 8.9 kDa oligopeptide (see Figure 1) we only observe fragments from the first 20 amino acids of the N-terminus. This is remarkable since we seldomly observe fragments arising from cleavages very close to the termini in the larger oligopeptides.

A third aspect of the fragmentation is the amount of sample needed to get fragments. At this moment 200 pmol is the minimum amount. This is a relatively large amount. The phenomenon that more fragments are observed when using larger amounts is known from Fast Atom Bombardment (FAB) mass spectrometry. It would be very useful to lower this amount needed.

In conclusion it can be stated that promising progress has been made. However, a lot of questions have to be answered. What is the upper mass limit for finding fragments? How is the internal energy gained in the ionization process distributed among the many oscillators? Can we influence the intensity of the fragment ions or the pattern of fragmentation? Can we learn more about the ionization/desorption process? Can we find methods using less sample and still find fragments? The majority of these questions seems to be closely related to applying a correctly chosen matrix.

References
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Figure 1: Plasma desorption mass spectrum of the YGC oligopeptide

![Mass spectrum](image)

Figure 2: Expanded low mass region of the YGC oligopeptide showing the sequence-informative fragmentation

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Table 1: Fragment ions from the YGC oligopeptide identified as the sequence ions mentioned. Peak numbers refer to Figure 1 and 2 - not shown in Figure 2

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ION/MOLECULE REACTIONS AND ENERGY DEPOSITION IN PYRENE STUDIED BY MS° EXPERIMENTS USING AN ION TRAP MASS SPECTROMETER.

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The well-known carcinogenic hazards posed by polycyclic aromatic compounds (PACs) has sparked a demand for new and reliable techniques for their characterization [1]. The quadrupole ion trap mass spectrometer (ITMS) includes unique capabilities for single ion selection and multiple activation steps (MS°) which provide methods for examining fragmentation pathways and energy deposition in PACs as well as elucidating their ion/molecule reactions [2].

Traditionally, the ITMS has been considered a device in which relatively low energy collisions occur. However, the structural stability of PACs necessitates that relatively large internal energies be supplied to observe fragmentation. Through the MS° capability of the ITMS, we have shown that large amounts of energy can be deposited in a sequential manner. As a result of these experiments, extensive fragmentation occurs to give dissociation reactions such as:

\[
\begin{align*}
\text{C}_{16}\text{H}_{10}^+ & \rightarrow \text{C}_\text{8} \text{H}_6^+ \rightarrow \text{C}_\text{8} \text{H}_4^+ \rightarrow \text{C}_\text{8} \text{H}_2^+ \rightarrow \text{C}_\text{6} \text{H}_2^+ \rightarrow \text{C}_\text{4} \text{H}_2^+ + \text{C}_\text{2} \text{H}_4^- \\
\end{align*}
\]

The activation energies for this process, approximated from \( \Delta H \) for each dissociation reaction, are \( 7.2 \pm 0.7, 0.6 \pm 1.3, 8.0 \pm 1.0, 3.9 \pm 0.9, 2.7 \pm 0.4 \) and \( 6.5 \pm 0.4 \) eV, respectively. Although each individual dissociation step has an activation energy < 8.0 eV, the total energy required to produce \( \text{C}_\text{8} \text{H}_4^+ \) by this route is \( 29 \pm 2 \) eV (the sum of the \( \Delta H \) values). An activation time of 50 msec at an ac amplitude of 1000 mV\( _{pp} \) \( (q = 0.26) \) was typically used. When unusually high activation conditions are applied \( (10 \ V_{pp} \ for \ 100 \ msec) \) to the molecular radical cation \( \text{M}^+ \), direct formation of the fragment ion \( \text{C}_{10} \text{H}_4^+ \) is seen (Fig. 1) although the dissociation efficiency for this process is only 7%. This process requires an energy between 17 and 22 eV, depending on the neutral losses observed. The base peak \( \text{C}_\text{16} \text{H}_4^+ \) is formed by loss of 6H from the molecular ion.

Fragmentation pathways of PAHs can also be assessed by utilizing the MS° capability of the ion trap. The fragments observed are similar to those seen with one-step high energy processes [3]. However, through the use of multiple activation and dissociation steps of the ITMS, the origin of these fragments can also be determined. Neutral losses common to all PAHs \( (e.g. \text{H}, \text{H}_2, \text{C}_\text{2} \text{H}_4, \text{C}_\text{3} \text{H}_6) \) were observed for the pyrene system and some fragment ions could be accessed by a number of different pathways. The longest sequence of dissociations performed on pyrene involved nine separate isolation and activation steps, producing \( \text{C}_\text{8} \text{H}_2^+ \) \( (m/z \ 84) \) from \( \text{C}_\text{16} \text{H}_4^+ \) \( (m/z \ 202) \).

Ion/molecule reactions of the pyrene molecular radical cation, \( \text{M}^+ \), the \( \text{(M-H)}^+ \) ion and the \( \text{(M-H}_2^2) \) ion, as well as the corresponding \( \text{C}_\text{16} \text{D}_{10}^+ \) ions, were investigated to further characterize ionized pyrene. The \( \text{C}_{16} \text{H}_{10}^+ \) species showed no reactivity toward the neutral reagents pyrene, methyl iodide, ethyl iodide, isoprene and 2-iodopropane. This is attributed to delocalization of the radical cation throughout the aromatic system. However, the \( \text{C}_{16} \text{H}_8^+ \) ion is an even electron species with a more localized charge site. It behaves much like the phenylium ion, showing high reactivities with the above reagents and giving predictable products. Consequently, the \( \text{(M-H}_2^2) \) ion shows intermediate reactivity in accordance with its odd electron character containing a localized charge and radical.

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Addition of isoprene to the (M-H)^+ and (M-H2)^+ ions of pyrene occurs via cycloaddition with a characteristic fragmentation by methyl radical loss from each adduct ion. Evidence obtained using d0-pyrene indicates that the adduct of (M-H)^+ fragments via methyl loss to give an ionized indene-type structure. Figure 2 suggests that the first CH3 loss does not come from the isoprene portion of the adduct ion, but from within the aromatic ring where H/D exchange can readily occur.

Addition of methyl iodide, ethyl iodide and 2-iodopropane to the (M-H)^+ ion of pyrene occurs through the iodine atom (C6H5-I^+). The methyl iodide adduct fragments by loss of I and CH3 to give methyl pyrene and iodopyrene product ions. CAD studies on the d10-iodopyrene ion (Fig. 3a) gave loss of I, indicative of this bridged-type structure. Reactions of ethyl iodide and 2-iodopropane with the (M-H2)^+ ion give primarily a HI addition product, together with small contributions from the intact adduct ion. In this case, the alkyl halide and the HI molecules add across the carbon-carbon bond rather than forming an iodine bridged adduct as indicated by the CAD products of the (C16D8 + HI)^+ ion (Fig. 3b). This data shows loss of I with no HI or H' loss.

An experiment in which the (2M-H)^+ adduct, C26H19^+, is converted to C22H5^+ in nine consecutive isolation and activation steps (MS10) without carbon-carbon cleavage, exemplifies the intriguing ion chemistry as well as the stability of the carbon skeleton of the adduct ions.

HIGH MASS-RESOLUTION USING A QUADRUPOLE ION TRAP MASS SPECTROMETER

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With recent developments in ionization techniques which transform molecules of high molecular masses into gas phase ions, mass spectrometry is gaining prominence as a method for biomolecule characterization. The quadrupole ITMS has been used to produce high sensitivity MS/MS spectra on peptides of molecular masses up to 3000 Da. To enhance the capabilities of this instrument, an offset DAC/attenuator network was constructed to slow down the scan rate when the ion trap is operated in the mass-instability mode of operation with resonance ejection \(^{1/2}\). Figure 1 shows that the unresolved isotopes of the \((M+H)^+\) region of Substance P (MW 1347) become more than unit resolved with an attenuation of 40x, and a final resolution of 350,000 (FWHM) is observed on the \(^{13}\text{C} (M+H)^+\) isotope (Figure 1d). As seen, the spacings are not equivalent between the isotopes. This is due to space charge effects which greatly influence the ejection process (Figure 2). This experiment was done using Substance P with an attenuation of 700x, and a change of only 1 ms in the ionization period. Space charge can be minimized by using a short ionization period and low He pressures. Similar high resolution results (Figure 3) have been obtained on another peptide Gramicidin S (MW 1140).

Due to instability of the peak position when the scan ramp is attenuated by 200 times or more, spectra can only be recorded under single scan conditions. To improve the quality of spectra and provide a means of off-line alignment of peaks for signal averaging and filtering, a data acquisition system has been implemented which samples the pre-amplified signal from the ITMS electronics. Due to differences in the sampling rate of the Keithley analog-to-digital converter and the scan rate of the drive frequency of the ion trap, it was necessary to preprocess the analog signal via a sample-and-hold filter prior to A/D conversion. Using this data system, the Ca\(^{11}\) cluster ion at \(m/z\) 3510 was studied (Figure 4). A marked increase in resolution occurs due to the lack of isotopic ions. A resolution in excess of \(10^6\) (Figure 4d) is achieved when the scan rate is slowed by a factor of 333 times (2000X attenuation divided by 6x mass range extension). The shape of the peak for this ion is not distorted with changes in the scan rate.

References
Figure 1

Figure 2

Figure 3

Figure 4
A sampling system using mass flow controllers has been developed for the introduction of gases at atmospheric pressure into the Finnigan ion trap mass spectrometer. This system is similar in concept to other atmospheric sampling systems that use capillary tubing or a single leak valve for pressure control. However, the mass flow control system described here has two distinct advantages over these methods. (1) Gas flow into the ion trap is regulated. This is especially important when the pressure of the sample gas is not constant, for example, when gases are produced by a chemical process in a closed or flowing system. (2) A second mass flow controller has been incorporated to introduce a gas which serves as an internal standard. We have observed substantial time-dependent decreases in ion trap response when monitoring gases in real-time (Fig. 1). In such cases, the internal standard can be used for abundance correction with dramatic increases in accuracy and precision.

Typical flow rates through the mass flow controllers are 30-40 cm$^3$/min. A constant pressure of about 10 torr in a differentially pumped interstage region results from these flow rates. Helium buffer gas is introduced directly to the ion trap vacuum manifold through an independent leak. In these experiments, the optimum helium partial pressure is 5 x 10$^{-4}$ torr and sample partial pressure is 1.3 x 10$^{-6}$ torr. A gas splitter is used to present sample and internal standard directly to the orifice of the sample introduction valve. Sample and internal standard are then directed into the electrode assembly of the ion trap through a modified sample entry port. Data were acquired using the automatic gain control function (AGC) of the ion trap mass spectrometer.

Argon and krypton have been assessed as internal standards in combination with CF$_3$Br and C$_2$HCl$_3$ as sample test gases. Argon ions at m/z 40 did not present mass interference to ions of interest in these experiments. However, under other conditions, ionized argon may present an interference with ions of analytical significance and has only a single major isotope to use as an internal standard, which leads to large changes in sample/standard ion intensity ratios (Fig. 2). Krypton was therefore evaluated as an internal standard. The krypton isotopes and their naturally occurring abundances are at m/z 78 (0.35%), m/z 80 (2.25%), m/z 82 (11.60%), and m/z 84 (57.00%). Using these isotopes for standardization, the krypton internal standard can cover a wide concentration range with a sample/standard ion intensity ratio near unity (Fig. 3).

No problems were observed as a result of charge exchange reactions. The limit of detection for trichloroethylene in air determined by exponential dilution is less than 10 ppm with the differentially pumped direct sample introduction system. The limit of detection for CF$_3$Br in N$_2$ is less than 40 ppm and is limited by background. The usable calibration range for CF$_3$Br spans from less than 10 ppm to 2% relative concentrations in air. Internal and external precisions for CF$_3$Br calibrations are 4.5% and 5%, respectively, for concentrations greater than 10 ppm. Experiments with various permanent gases and C$_1$ to C$_6$ aliphatic hydrocarbons are in progress to further evaluate this system.
Figure 1. Ion trap response to CF$_3^+$ has been observed to decrease with time.

Figure 2. Using an internal standard improves the linearity and slope of the calibration curve for CF$_3^+$.

Figure 3. Using several Kr isotopes for standardization further improves calibration linearity, especially over wide concentration ranges.
Relative Ion Trap Storage Efficiencies of Ions Up to m/z 1500 By Capillary GC/MS

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INTRODUCTION - There has been increased interest in recent years concerning interfacing external ion sources to the ion trap mass spectrometer (ITMS). This is primarily due to the potential for sensitive sequencing of biologically important compounds such as peptides, proteins and oligonucleotides using the MS" capabilities of the ITMS. Storage of ions injected from external sources has been reported out to m/z 50,000 on an ITMS (1). However, the storage efficiency of ions above the "normal" 650 Da mass range of the ITMS has not been addressed. Therefore, the storage characteristics of "high mass" ions were investigated independently of external ion sources by using volatile "high mass" compounds.

EXPERIMENTAL - Trisperfluoroheptyltriazine (MW 1185) and trisperfluorononyltriazine (MW 1485) were dissolved equimolar with propylbenzene (MW 120) and decylbenzene (MW 218) (Fig. 1) and introduced into the ITMS by capillary gas chromatography (GC) (15 m x 0.25 mm i.d., 0.25 micron film, DB-1, Injector port 250°C, 5 pmol on column). The scan editor software was used to vary the rf-voltage level at the electron ionization pulse in each separate experiment. The "high mass" work was done using z-axis ejection (6 V) at a $q_z$ value of 0.30067.

RESULTS - The alkylbenzenes were used to bracket the triazines by retention time and to serve as positive controls for "normal" ion trap operation since their $q_z$ values are high relative to those of the triazines. All test compounds were introduced via capillary GC and electron ionization was used to create the ions within the electric field. The trapped ions were then scanned out and the total ion current was used to accurately measure the area under the gaussian GC peaks that appear at unique retention times. The GC peak areas for a family of linear alkylbenzenes run under "normal" Ion trap conditions were consistent over an rf level range corresponding to a cut-off mass of 8 - 45 Da (Figure 2). Below 8 Da, the field collapses and above 45 Da apparently the electron energy and the trajectory of the electrons become unfavorable. In both cases the capillary GC peak areas decrease sharply. This range of rf levels was considered to be the most favorable region to make and store ions in the trap. This working range of optimal rf levels corresponds to $q_z$ values for the triazines ranging from 0.005 to 0.035 and $q_z$ values for the alkylbenzenes ranging from 0.033 to 0.341.

The mixture of alkylbenzenes and triazines run at an ionization rf level of 30 Da showed slightly greater GC peak areas for the triazines than the alkylbenzenes. At this rf level all compounds had $q_z$ values greater than 0.018. This indicates that the "higher mass" triazines are being stored efficiently and that they have slightly higher ionization cross-sections relative to alkylbenzenes. When the ionization rf level is dropped to 10 Da, the GC peak areas for the triazines are reduced by at least an order of magnitude relative to the alkylbenzenes which stay within 70% of their initial peak areas. Clearly, the $q_z$ values (less than 0.008) of the triazines are too low and they are not efficiently stored under these operating conditions (Fig. 3). Therefore, for optimal sensitivity we suggest $q_z$ values greater than 0.02 during injection of ions in the 1500 Da range.

Once "high mass" ions have been created and efficiently stored in the ion trap they can be mass analyzed using z-axis ejection at different $q_z$ values (0.30067 was used for all of these experiments). Operating beyond the "normal" mass range of the ion trap (650 Da) using z-axis ejection techniques requires calibration of the mass range. The mixture of triazines and alkylbenzenes were used to calibrate the ITMS out to m/z 1500. The intense well-known ions of the triazines and the alkylbenzenes were observed in the z-axis ejected mass spectrum at approximately one-third their normal m/z values. A correction factor (0.91/0.30067 = 3.03) was applied to these m/z values and the approximate m/z values were plotted against the known m/z values for each of the appropriate ions. The line has a slope of 1.011 and a y-intercept of -2.1698 with an excellent correlation coefficient of 1.000.

The calibration line was then used to measure the mass spectrum of perfluorotributylamine (PFTBA, FC-43), with its ions z-axis ejected at $q_z = 0.30067$. Figure 4 shows the "calibrated" mass spectrum of PFTBA under the operating conditions described above. The measured m/z values are as much as 3 Da off from the expected values. We hypothesize that this is due to a limitation in the ITMS software package. The mass range has been tripled over the same number of DAC steps, and masses are still assigned to 1 Da "buckets." These errors are compounded by back multiplication.
Large Scale Computer Simulation of the Quadrupole Ion Trap Mass Spectrometer

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Computer simulation of the quadrupole ion trap has long been a tool for analyzing the performance of this device [1]. Programs which have been developed in the past have been effective at determining the stability of a limited number of ions under static conditions of constant RF amplitude [2]. Some programs have included models of ion-neutral collisions as a means of simulating the effects of a buffer gas present in the trapping space [2]. Recently computer models of ion motion under the influence of auxiliary (AC) potentials applied to the end-cap electrodes have been used to study the nature of resonance excitation and resonance ejection [3]. To better understand how changes in device operation will affect performance, a new program has been written in our group to simulate the ion trap on a large scale which can produce simulated mass spectra, as well as other information. The program implements a set of "scanning" functions for the amplitude and frequency of each of the potentials making up the electric field: RF, DC, and the Auxiliary (AC) potential. Utilizing large virtual memory computers with vector processors a goal of \(10^6\)-\(10^7\) ions is possible.

In the results reported here, the simplest version of the program has been used. It uses a Taylor Series expansion of the Mathieu equation to compute the ion trajectories, and buffer gas collisions have been ignored. To study the effects of auxiliary fields, a dipole field term has been added to the Z component of the electric field equation. The program requires an ion population be "created" by the user prior to execution so changes in operation can be observed on the exact same ion population. A number of ions of any m/z value may be generated according to user specified distributions for initial position and velocity.

The program has been used to study the effects of scan speed on resolution and to observe mass shifts which occur during axial modulation mass range extension. Figure 1 shows the simulated (top) and experimental (bottom) mass spectra of the molecular ion region (M+H)+ of Substance P, a peptide with mass 1347, using the normal mass instability scan speed of \(\sim 62400\) V/sec and a 2.25X mass extension. A mass shift of approximately 2.5 amu was corrected in the experimental data, and the simulated data was corrected by the same amount. In Figure 2, the exact same ion population as was used in Figure 1 is now scanned at 1/10 the normal speed. As indicated a mass shift is observed when axial modulation is used to extend mass range. The nature of this mass shift and its causes are not well characterized, preventing accurate mass assignment at high mass (large mass range multiplications). Figure 3 shows a both the experimental and simulated mass shifts for the CsI cluster m/z 912.3, as a function of mass range multiplication. The simulation matches the experimental data for the lower multiplication factors despite not modeling field distortions, space charge effects or the buffer gas.


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Figure 1. Simulated (top) and Experimental (bottom) mass spectra of Substance P, normal scan rate, 2.25X mass range extension.

Figure 2. Simulated (top) and Experimental (bottom) mass spectra of Substance P, 1/10 the normal scan rate, 2.25X mass range extension.

Figure 3. Simulated vs. experimental mass shifts as a function of the mass range multiplication factor.
The isolation of ions is recognized as an important step in many of the experiments that can be performed with the quadrupole ion trap mass spectrometer. Selective mass storage (SMS) at the apex of the stability diagram has been employed to isolate a parent ion prior to additional stages of mass spectrometry, including collision induced dissociation and ion/molecule reactions (1). Also, notable examples of SMS to reduce space-charge conditions in the trap have shown improved results for a variety of analytical studies, including MS/MS of biological extracts (2) and isotopically labeled internal standards (3).

The tandem-in-time nature of the ion trap allows us to change the analyzer voltages and the associated trapping field while an ion is held in the trap. A new mass isolation technique, dubbed "Two-step isolation", takes advantage of the tandem-in-time nature of the ion trap by separating the mass isolation process into two steps that are performed sequentially in time. In general, an ion is isolated by first applying analyzer voltages to the trap that produce unstable conditions for ions that have an m/z greater than the ion that is to be isolated. Next, the analyzer voltages are changed so that all ions with an m/z less than that of the ion of interest follow unstable trajectories. The combination of these two steps results in the isolation of a single m/z in the ion trap.

We have performed a comparative study of SMS and Two-step Isolation which reveals that the Two-step method is 1.5 times more efficient than SMS. Although the Two-step isolation scan function is more complicated than the SMS isolation scan, it requires less time to set up and is much easier to tune due to the separable nature of the method. Evaluation of the $\beta_1=0$ and $\beta_2=0$ edges of the stability diagram show that the best stability edge for eliminating higher mass ions without loss of the ion of interest is $\beta_1=0$ at $q=0.7$. We also observe that axial modulation offers slight improvements in the "sharpness" of the $\beta_2=1$ edge. Two new figures of merit were introduced that (a) measure how efficiently the ion of interest is stored and (b) assess how effectively ions other than the ion of interest were ejected.

The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Two-Step Isolation Scan

Mathieu Stability Diagram for m/z=157
in RF/DC Space

![Graph showing Mathieu Stability Diagram](image)

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Introduction

The Ion Trap Mass Spectrometer is a very sensitive instrument, but trace detection can be severely limited by the presence of abundant matrix ions. Accumulation of unwanted ions can lead to limited ion accumulation time due to early onset of space charge. Previous studies [1] have shown that removal of unwanted ions during accumulation of ions injected from an external ion source enhances analyte signal by allowing longer accumulation times before onset of space charge. We have investigated two selective ion accumulation techniques that eject unwanted ions during electron ionization within the trap. It was our intention to identify methods which would yield the maximum usable analyte signal from a complex mixture.

Methyltestosterone was chosen as a model of a complex mixture because of its complex mass spectrum. A relatively low abundant ion was chosen as the "analyte" ion while all others were considered unwanted ions and served as the "matrix". The two mass-selective ion accumulation techniques investigated were DC ejection and DC/Resonant ejection.

Results

**DC Ejection**

While operating at a \( q < 0.85 \) for the ion of interest, we applied either a +DC or -DC voltage to the ring electrode so that ionization would be performed at either the \( \beta_x = 0 \) or \( \beta_y = 1 \) stability edge. It is possible to selectively store any range of ions in the spectrum by simply moving along either stability edge. Figure 1 compares the full mass spectrum of methyltestosterone to a spectrum acquired at \( q = 0.8 \) and \( a = 0.3 \) (\( \beta_z = 0 \) stability edge). Figure 2 shows results of accumulation of m/z 41 while a DC voltage was used to eject all ions above m/z 50. The full mass signal for 41+ shows a total loss of resolution above 1 ms ionization time while DC ejection allows 50 ms ionization time without loss of resolution and a 20 fold increase in ion signal with +DC ejection and 10 fold increase with -DC ejection. Accumulation of ions above m/z 100 is less efficient with this technique. Accumulation of m/z 199, for example, requires a 15 fold increase in ionization time in order to achieve a 2 fold increase in the full scan 199+ ion signal. This is believed to be due to the higher voltages required to reach a stability edge for higher m/z ions. High voltages applied during ionization are believed to disrupt the ionization process by altering electron energy and electron trajectory within the trap. Although some signal is lost, a linear increase in ion signal is obtained with increasing ionization time.

**DC/Resonant Ejection**

While ionizing at \( q = 0.2 \) for the ion of interest, a supplementary excitation signal \((6V_{pp})\) was applied across the endcap electrodes to eject lower mass ions while a +DC voltage was applied to the ring electrode to eject higher mass ions. We investigated two frequency-swept resonant ejection techniques: frequency scanning, which is a single linear scan of the frequency range and broadband ejection, which is a customized frequency sequence that repetitively sweeps the frequency range. Figure 3 compares efficiency of resonant ejection for both techniques by scanning the frequency range between 375 kHz and 75 kHz (m/z 33 to m/z 164). Since an ion's
resonant frequency is scanned repetitively during ionization with the broadband technique, the unwanted ions are only allowed to accumulate after the last scan. Figure 4 compares results of accumulation of 199+ ion with DC/Resonant broadband ejection and with DC ejection. DC ejection exhibits a linear response up to 50 ms while DC/Resonant ejection shows non-linearity at high ionization times. This is believed to be due to the limited amplitude offered by the internal frequency synthesizer (6V_p-p). Although DC ejection yields a higher signal for m/z 199, DC/Resonant ejection may be more useful for accumulating higher m/z ions since much lower voltages are used with this method.

EXTENDING THE DYNAMIC RANGE OF THE QUADRUPOLE ION TRAP FOR GC/MS ANALYSES EMPLOYING ISOTOPICALLY LABELLED INTERNAL STANDARDS

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Introduction
The extreme sensitivity and versatility of the quadrupole ion trap mass spectrometer (ITMS) would appear to make GC/ITMS the method of choice for GC/MS trace analysis. However, there have been limited applications of this sensitive mass spectrometer to quantitative GC/MS analyses employing isotopically labelled internal standards (IS). An analyte and its isotopically labelled IS typically coelute, often producing space charge conditions which may cause loss of sensitivity, mass resolution, and degradation of spectral quality. This typically limits the linear dynamic range (LDR) of these analyses to two orders of magnitude.

Recently we have developed a new ion trap scan method which employs alternating mass selective storage (RF/DC isolation) for ions of an analyte and its coeluting isotopically labelled IS in successive scans. This work presents the results of this method and compares them to results obtained using a similar method which utilized a supplementary FORTH program to control alternating RF/DC isolations [1,2]. The effects of axial modulation on the LDR for these types of analyses are also presented.

Experimental
All experiments were performed on a Finnigan MAT quadrupole ion Trap Mass Spectrometer interfaced to a Varian 3300 Gas Chromatograph via a specially designed, resistively heated transfer line probe. Compounds were dissolved in o-xylene and serial dilutions performed. The GC effluent was ionized by El and m/z's 163 and 167 were monitored for the analyte and IS, respectively.

Results and Discussion
The results of linear regression analyses (peak area ratio 163+/167+ vs. amount ratio d4/d4) with and without axial modulation are shown in Table 1. Use of axial modulation provides excellent linear correlations (r^2 = 0.9994) and precision (RSD's of triplicate injections <6%) over three orders of magnitude. Because of these improvements, axial modulation was used in subsequent studies involving RF/DC isolation.

Table 2 shows a condensed version of the new RF/DC isolation scan function developed for these studies. It requires no supplementary FORTH program to control alternating between analyzing an analyte and IS in successive scans. The RF timing traces of Figure 1 show the acquisition rate of this method being 8 times faster than the previously used method [1,2]. Table 3 compares results of linear regression using this method with the previously used method. The new method provides excellent linear correlations (r^2 = 0.9991) and precision (RSD's of triplicate injections <10%) over four orders of magnitude. The improvements over the previous method are attributed to the ability to acquire more data points over a GC peak which results in more reproducible peak shapes and areas for quantitation.
Table 1: Summary of Linear Regression Results Revealing Improvements with Use of Axial Modulation

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<td>0.9794</td>
<td>1.082</td>
</tr>
<tr>
<td>4</td>
<td>0.7224</td>
<td>0.284</td>
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</table>

Table 2: Condensed Listing of ITMS Scan Function for Combined RF/DC Isolations

<table>
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<tr>
<th>Component Analyzed</th>
<th>Scan Table (a)</th>
<th>Time (ms)</th>
</tr>
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<tbody>
<tr>
<td>Trigger</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>1st Ionization Pulse</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>1st Data Acquisition Ramp</td>
<td>2.70</td>
<td></td>
</tr>
<tr>
<td>(m/z 163)</td>
<td>(m/z 161 $\rightarrow$ m/z 165)</td>
<td></td>
</tr>
<tr>
<td>RF Shut Down</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Trigger</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>2nd Ionization Pulse</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>2nd RF/DC Isolation</td>
<td>2.70</td>
<td></td>
</tr>
<tr>
<td>(m/z 167)</td>
<td>(m/z 165 $\rightarrow$ m/z 169)</td>
<td></td>
</tr>
<tr>
<td>RF Shut Down</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.74</td>
<td></td>
</tr>
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</table>

*Includes application of axial modulation

Table 3: Summary of Linear Regression Results Comparing Methods of RF/DC Control

<table>
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<tr>
<th>Orders of Magnitude</th>
<th>$r^2$</th>
<th>slope</th>
<th>RSD</th>
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<tr>
<td>2</td>
<td>0.5492</td>
<td>0.679</td>
<td>&lt;15%</td>
</tr>
<tr>
<td>3</td>
<td>0.9255</td>
<td>0.152</td>
<td>&lt;19%</td>
</tr>
<tr>
<td>4</td>
<td>0.9779</td>
<td>0.106</td>
<td>&lt;19%</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Combined Scan Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>
EFFECTS OF EXPERIMENTAL PARAMETERS ON ION STORAGE IN A QUADRUPOLE ION TRAP MASS SPECTROMETER

K.J. Hart, Scott A. McLuckey and Gary L. Olish
Oak Ridge National Laboratory, P.O. Box 2008
Oak Ridge, TN 37919-6365

Several empirical and simulation studies have been undertaken to increase our understanding of the operation of the Ion Trap Mass Spectrometer (ITMS). In particular, the poor ion yields obtained when attempting to mass-selectively accumulate ions of a particular m/z using combined RF and DC voltages applied to the ring electrode during ionization was investigated. The experimental studies compared the ion abundances (Σ m/z 117 thru m/z 123) observed in the spectrum obtained for N,N-dimethylaniline (DMA) using electron ionization (EI) and photoionization (PI). The abundance of the DMA ions decreased by 85% to 90% as the RF voltage during the ionization pulse was changed from a low mass cutoff of 30 (q[121] = 0.225) to a low mass cutoff of 110 (q[121] = 0.825) as shown in Figure 1. The DC component of the voltage applied to the ring electrode was zero. The abundance of the DMA ions observed as the DC voltage was changed from -100 V to +280 V at an RF level of 105 also varied as shown in Figure 2. Note that the maximum signal for PI was 0 V while the maximum signal for EI was +190 V. Originally, it was thought that the differences observed between PI and EI might be due to an effect on the electrons by the quadrupole field that would not be observed on photons. One factor that complicated this comparison was the orientation of the ionizing beam in the EI (oriented axially - z) and PI (oriented radially - r) experiments. An electron gun assembly was devised that allowed radial injection of electrons into the ion trap. The results for axial and radial injection of electrons versus RF voltage and DC voltage are shown in Figures 3 and 4, respectively. Note that these results show the decrease in abundance with increasing RF level (ionization) as was shown in Figure 1. The data shown in Figure 4 indicate that the difference between the EI and PI data lies in the orientation of the ionizing beam and not with an effect on the electron.

Simulation of the ITMS using a modified version of the SIMION program and the included ion trap simulation was used to estimate "effective ionization path lengths" and "effective ionization volumes" within the trap where neutral molecules at thermal temperatures can be ionized and remain trapped. Ions which are formed outside of this area are lost from the trap even though the a,q parameters which determine the m/z values that are stored have been appropriately selected. These ions have been termed "quasi-unstable" [1]. Other ion loss mechanisms such as scattering, charge transfer, ion-molecule reactions, non-linear resonances and self-emptying were not considered. The sample pressure and ionization times for the experimental data were selected so that complications due to space-charge should be small. A plot of radial and axial effective path length estimated from the SIMION simulations is provided in Figure 5. The effective axial path length decreased substantially (approximately 72%) from an RF level of 30 to an RF level of 110 while the radial path length changes very little (approximately 4%). The ionization volume (calculated as a cylinder - πr²z) decreased 74% over the same RF range. The cause of the decreased abundance observed for the DMA ions observed using PI (quite similar to that observed for EI) may be due to scattering of the laser radiation in the trap and or emission of secondary electrons and subsequent ionization of DMA within the effective ionization volume. Both of these ionization mechanisms would account for a decreased abundance of ions due to the smaller ionization volume at the two RF voltages. An additional 12% ion loss was observed when an ionization RF voltage corresponding to a low mass cut-off of m/z 30 was used with a ramp up to m/z 110 and an analysis ramp from m/z 110 to m/z 140.

For the case where the trap was set to store only one specific m/z, the calculated effective path length and ionization volume decreases to virtually zero. This includes both the upper (-DC) and lower (+DC) apexes of the ion trap stability diagram. It is possible, of course, to decrease the DC voltage and accept less than perfect isolation of specific m/z.

Research sponsored by the U.S. Department of Energy, Office of Basic Energy Sciences, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc. and an appointment to the U.S. DOE Laboratory Cooperative Postgraduate Research Training Program administered by Oak Ridge Associated Universities.
Comparison of El and PI versus RF Level During Ionization Pulse

Comparison of Axial and Radial Injection of e⁻ Versus RF Level

Comparison of El and PI Versus DC Voltage During Ionization Pulse

Comparison of Axial and Radial Injection of e⁻ Versus DC Voltage

Axial and Radial Effective Path Lengths

Effective Ionization Path Length (cm)
"SPONTANEOUS" CLUSTER DECOMPOSITION IN A QUADRUPOLE ION TRAP

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John E. Bartmess
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Cluster ions normally found in high pressure ion sources are not usually observed under chemical ionization conditions in the quadrupole ion trap. This is mainly due to the lower pressures in the ion trap which affect third body stabilization. But, even for cluster ions injected from an external source, decomposition is observed. By injecting cluster ions of known stability and measuring the decomposition rate as a function of pressure and time, we can determine what affects various parameters have on the internal ion temperatures in the quadrupole ion trap.

The desolvation experiments were carried out by injecting ions formed via electrospray into the quadrupole ion trap. The atmospheric sampling glow discharge ionization (ASGDI) source served as the interface between atmosphere and the vacuum chamber. Protonated water clusters were formed by spraying distilled water at a flow rate of 1 μL/min and protonated methanol clusters were formed by spraying HPLC grade methanol at 1μL/min. Since fragmentation of ions upon injection into an ion trap has been observed in our lab as well as others, we needed to determine if ions are "excited" during injection. Therefore, the cluster ion mass spectra from the ion trap was compared to the cluster ion mass spectra from a quadrupole mass filter using the same interface and electrospray conditions. The results showed that, under the ion injection conditions used in this study for both the water and methanol clusters, significant internal excitation did not occur during injection. The clusters, therefore, acquired the necessary energy for dissociation after they were trapped.

The major variables found to affect the appearance of the mass spectra for the cluster ions are time and pressure. The pressure studies, illustrated in Figure 1, show that at higher helium bath gas pressures (1 mtorr) rapid desolvation occurs due to higher collision frequencies. At relatively short delay times, the highest protonated methanol cluster observed is the tetramer. With no helium bath gas present which corresponds to a pressure of 2 x 10^-5 torr, lower collision frequencies favor the appearance of higher protonated species (hexamer) but overall shows more extensive desolvation, dissociating to \(\text{H}_3\text{O}^+\) compared to the protonated methanol dimer with 1 mtorr of He.

To calculate the desolvation rate constants, the cluster ion of interest was isolated and the rate of loss was monitored by varying the time delay. Figure 2 shows the plot of the negative logarithm of the relative water tetramer signal as a function of delay time indicating a linear decay expected from pseudo first-order kinetics. The overall decomposition (desolvation) rate is actually second order since the rate determining step is expected to be the rate of energy transfer via collision rather than the rate for unimolecular decay of the cluster. Hence, the bimolecular rate constant for these cluster ions is determined by dividing the slope of the line by the neutral number density. For each cluster ion studied, the desolvation rate constant decreased with increasing bath gas pressure. As shown in Figure 3, the desolvation rate constant for the water tetramer has an inverse relationship with the log of the bath gas pressure. This is attributed to the rate constant increasing with ion internal temperatures.

Based on the bimolecular (desolvation) rate constants calculated from the kinetics experiments, an internal temperature for these cluster ions was assigned using the thermochemical parameters for these desolvation reactions reported by Kebarle and co-workers(1) as well as others(2). Table 1 lists the dissociation threshold temperature and the calculated internal temperatures for the cluster ions at given pressures. The internal temperature was calculated by the equation:

\[ T = -\Delta H/R \ln(k_{\text{obs}}/k_{\text{inf}}) - \Delta S \]

where \(k_{\text{inf}}\) is the standard trajectory rate constant(3).
Table 1

Calculated Internal Temperatures for Cluster Ions

<table>
<thead>
<tr>
<th>P(minorr)</th>
<th>T(K)</th>
<th>T'</th>
<th>q'</th>
</tr>
</thead>
<tbody>
<tr>
<td>(MeOH)H⁺</td>
<td>0.74</td>
<td>401</td>
<td>537</td>
</tr>
<tr>
<td>(MeOH)H⁺</td>
<td>0.02</td>
<td>444</td>
<td></td>
</tr>
<tr>
<td>(MeOH)H⁺</td>
<td>0.74</td>
<td>433</td>
<td></td>
</tr>
<tr>
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<td>0.097</td>
<td>504</td>
<td></td>
</tr>
<tr>
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<td>1.2</td>
<td>581</td>
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<tr>
<td>(MeOH)H⁺</td>
<td>0.74</td>
<td>585</td>
<td>1083</td>
</tr>
<tr>
<td>(MeOH)H⁺</td>
<td>0.087</td>
<td>605</td>
<td></td>
</tr>
<tr>
<td>(MeOH)H⁺</td>
<td>0.02</td>
<td>744</td>
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</tr>
<tr>
<td>(H₂O)H⁺</td>
<td>0.74</td>
<td>565</td>
<td>469</td>
</tr>
<tr>
<td>(H₂O)H⁺</td>
<td>0.087</td>
<td>591</td>
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</tr>
<tr>
<td>(H₂O)H⁺</td>
<td>1.2</td>
<td>402</td>
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<tr>
<td>(H₂O)H⁺</td>
<td>0.74</td>
<td>448</td>
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<tr>
<td>(H₂O)H⁺</td>
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<td>657</td>
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</tbody>
</table>


Ion-Locked Cyclotron Resonance: 
A Means for Instantaneously Changing Ion Cyclotron Orbital Radius 

Ruldan Chen†, Alan G. Marshall‡,# and Mingda Wang‡

Departments of Chemistry† (and Biochemistry‡), The Ohio State University, 120 West 18th Avenue, Columbus, OH 43210
‡Walnut Creek Instrument Division, Varian Associates, 2700 Mitchell Drive, P.O. Box 9016, Walnut Creek, CA 94598-0916

The central Lorentz magnetic force, \( q v \times B_0 \), acting on an ion of velocity, \( v \), and charge, \( q \), moving in a static magnetic field, \( B_0 \), may be increased (or decreased) by adding a rotating electric field, \( E_{\text{eff}} \), directed radially inward or outward [i.e., along (or opposed to) \( v \times B_0 \)] (Fig. 1). The effective magnetic field is \( B_{\text{eff}} = B_0 \pm (E_{\text{eff}}/v) \), in which \( v \) lies in a plane perpendicular to \( B_0 \). Because the ICR orbital radius, \( r \), varies inversely with \( B_{\text{eff}} \), such a rotating rf electric field can be used to pulse the radius (and ICR orbital frequency, \( \omega_c = qB_0/m \)) effectively instantaneously to larger or smaller value, in an experiment we call "ion-locked" cyclotron resonance by analogy to "spin-locking" in NMR [2]. The "ion-lock" event sequence for Figure 1c is shown in Fig. 3.

Following a conventional coherent resonant excitation of ions of a given \( m/q \) ratio to a given radius by an rotating electric field, \( E = E_0 \exp(-i\omega_c t) \), the phase of the electric orbit field can be shifted by \( \pm 90^\circ \) with a concomitant increase in rf amplitude and frequency to "lock" ions into a smaller (Fig. 1c, 2a) or larger (Fig. 2c) radius orbit whose ICR orbit center is spatially displaced. In order to produce a \(-90^\circ\) phase shift, \( E_{\text{eff}} = E_0 \exp[-i(\omega_{\text{eff}} t + \pi/2)] \) for the ion-lock event, where \( E_{\text{eff}} >> E_0 \). Note that if \( E_{\text{eff}} \) is to be kept aligned along \( q v \times B_{\text{eff}} \), then the frequency of the rf electric field and the instantaneous ICR orbital radius must change from \( \omega_c \) and \( r_0 \) to \( \omega_{\text{eff}} \) and \( r_{\text{eff}} \) at the same instant that the rf phase is shifted by \(-90^\circ\):

\[
\omega_{\text{eff}} = \frac{qB_{\text{eff}}}{m} = \omega_c \left(1 + \frac{E_{\text{eff}}}{vB_0}\right) \quad \text{and} \quad r_{\text{eff}} = \frac{r_0}{1 + \frac{E_{\text{eff}}}{vB_0}}
\]  

The feasibility of the ion-lock experiment has been treated analytically as a function of \( m/q \), and supported by ion trajectory modeling, as will be reported more fully elsewhere [3]. Possible applications include: (a) rapid mass-selective ion ejection by \(+90^\circ\) shift in the phase of \( E_{\text{eff}} \) (Fig. 2c); (b) mass-selective shift in ICR orbital radius and ICR orbit center (Fig. 1c, 2a, 2c); and (c) increased ion energy for collision-induced dissociation by a combination (Fig. 2b) of a traditional excitation event followed by an ion-lock excitation event: e.g., \( E(t) = vB_0 \exp[-i(2\omega_c t + \pi/2)] + E_2 \exp(-i2\omega_c t) \), with \( E_2 << vB_0 \). This work was supported by N.S.F (CHE-9021058) and The Ohio State University [3].

References
Figure 1. The vectors, forces acting on ions and their corresponding ion trajectories in a homogeneous magnetic field (a) without an rf electric field; (b) during and after a traditional excitation event; (c) during an ion-locking event.

Figure 2. Ion trajectories during (a) an ion-lock event; (b) a combination of a traditional excitation event and an ion-lock excitation event; (c) an ion-lock ejection event.

Figure 3. The changes with time of (a) the ICR radius; (b) the magnitude of the rf electric field; (c) the phase of the electric field; and (d) the ICR frequency for an ion-lock experiment.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

ULTRAHIGH RESOLUTION FT/ICR MASS SPECTROMETER

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120 West 18th Avenue, Columbus, Ohio 43210
* On leave of absence from the Institute of Physics, University of Mainz, Germany.

In this abstract, we describe our recently constructed ultrahigh-resolution FT/ICR mass spectrometer, and show two of the first spectra recorded with this instrument.

The ultrahigh vacuum needed for high mass resolving power is provided by a Perkin-Elmer TNB-X pumping station, comprising a sorption pump, ten ion pumps (300 L/s pumping speed) and four titanium sublimation filaments which provide increased pumping speed for removal of common residual gases. The entire vacuum chamber can be baked at 200 °C.

The vacuum system is mounted on a rail carriage riding on Thompson linear bearings. During bakeout it is possible to roll the vacuum system out of the Oxford horizontal-bore 7-tesla superconducting magnet (magnetic field homogeneous to within 1 part in $10^5$ over a $1" \times 1" \times 4"$ volume). A non-magnetic 1" cubic ion trap built of oxygen-free hard copper is designed to accommodate screens. When these are grounded they shield the ions from the effect of the trapping field so that the ICR frequency becomes virtually independent of orbital radius and trapping potential [1]. An electron gun for electron beam ionization is mounted in the fringe field (about 0.02 T) of the magnet, at a distance of ~1 m from the center of the ion trap. Moving the ion gun largely eliminates the transmission of noise from the electron-gun filament power supply to the detect plates of the ion trap. It also assures that the electron beam and ion trap are centered in the magnetic field and that the spread in initial radial position of the ions is minimized, because the electron beam is focused by the magnetic field.

The excitation and detection electronics are built around an Extrel 2000 data station. Performance of the data station was extended to the high ICR frequencies of low-mass isobars (e.g., ~27 MHz for $m/z = 4 \ u/e$ at 7 tesla), by addition of a PTS-40 frequency synthesizer (40 MHz), AR-25A100 high frequency power amplifier, homebuilt detection preamplifier, and associated TTL switches, filters, mixers, and buffers. For precision monitoring of doublets, dual-frequency excitation is generated by heterodyning sinusoids from the PTS frequency synthesizer and the Extrel data station. This excitation method can be followed by one of two detection schemes. The ion decay signal can be simultaneously heterodyned with the dual reference signals generated for excitation. Alternatively, cascaded heterodyning of the free ion decay signal can be performed by mixing it with the carrier signal from the PTS-40 and then mixing the produced difference signal with the Extrel 2000 reference signal. The data are then Fourier transformed and displayed by the Extrel 1280 computer. Details of these procedures will be described elsewhere [2].

In this way, the frequencies of the signals of ions of two different $m/z$ values can be made arbitrarily low and close together [2], thereby vastly decreasing the number of sampled data points without loss of precision in the determination of the resonant frequencies. For example, ICR signals (not shown) from $N^+$ and $N_2^+$ (frequency difference of 3.86 MHz) have been shifted to only 100 Hz apart, without the added noise produced by multiple-foldover techniques [3]. The performance of the system is illustrated in Figures 1 and 2. This work was supported by N.S.F (CHE-9021058) and The Ohio State University.
Figure 1. FT/ICR magnitude-mode mass spectrum of electron-ionized $^4\text{He}^+$ and $\text{D}_2^+$, based on a single time-domain free ion decay transient signal. The detected signal frequencies, 1007.6 Hz and 969.0 Hz (i.e., a frequency difference of 38.6 Hz), correspond to actual ICR frequencies of 27,031 and 26,859 kHz (i.e., a frequency difference of 172 kHz).

$\Delta m > 50,000,000$

$\Delta m = 19.5 \text{ nAMU} = 18.2 \text{ eV}$

$\frac{m}{\Delta m} > 200,000,000$

$\Delta v = 0.132 \text{ Hz (FWHM)}$

Resolving power is limited by the base pressure, measured as $3.6 \times 10^{-11}$ torr (gas constant of the Bayard-Alpert ion gauge set for m/z = 28 u/e).

References

HARTLEY/HILBERT TRANSFORM SPECTROSCOPY: ABSORPTION-MODE RESOLUTION WITH MAGNITUDE-MODE PRECISION

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Fourier transformation (FT) of an N-point time-domain discrete signal produces, after phase correction, two independent data sets: an N/2-point absorption spectrum, A(ω), and an N/2-point dispersion spectrum, D(ω), each with the same information content. Usually only A(ω) is kept. The dispersion-mode information has conventionally been recovered in either of two ways. [1] First, the N/2-point magnitude-mode spectrum, M(ω) = [(|A(ω)|^2 + |D(ω)|^2)^(1/2), offers a √2 improvement in precision compared with the original N/2-point absorption spectrum, but with poorer resolving power (factor of √3 to 2). Alternatively, zero-filling the initial time-domain data to 2N data points prior to Fourier transformation results in an N-point absorption-mode spectrum with the same peak width and peak-height-to-noise ratio as the original N/2-point absorption spectrum, but with a √2 improvement in precision. Thus, magnitude-mode display improves FT spectral precision [2] at the expense of a loss in resolving power, whereas zero-filling improves precision at the expense of having to store twice as many data points.

In this abstract, we describe a third method of recovering the dispersion information. Specifically, discrete Hilbert transform of D(ω) (obtained by discrete Hartley transformation [3] of N time-domain data) yields an N/2-point "pseudo-absorption" spectrum which may be added to the original A(ω) to yield an N/2-point "enhanced" absorption spectrum with the same peak width and same number of data points, but with peak-height-to-noise ratio improved by a factor of √2 over the original N/2-point absorption spectrum. In other words, the new procedure yields an FT spectrum with enhanced precision, without any attendant loss in resolving power (as for magnitude-mode display) and without any increase in the number of stored data points (as for zero-filling).

Figure 1 shows absorption-mode, magnitude-mode, and enhanced absorption-mode spectra for a simulated time-domain signal consisting of three damped sinusoids of relative amplitude, 10, 1, and 3. The peak corresponding to the weakest signal is much more easily distinguished from the baseline noise in the enhanced absorption-mode signal. (Magnitude-mode offers the same ratio of peak height to baseline noise standard deviation as does enhanced absorption-mode, but the root-mean-square magnitude-mode noise is √2 higher, making the signal peak harder to recognize visually.)

Figure 2 shows the method applied to actual FT/ICR experimental data for N₂⁺ produced by electron ionization (Extral FTMS-2000, single-frequency on-resonance excitation, heterodyne-mode detection at 129 kHz bandwidth to yield 8K time-domain data). The narrower peak width for the enhanced absorption-mode compared to magnitude-mode is clearly evident.

This work will be reported more fully elsewhere, [4] and was supported by N.I.H. (GM-31683) and Ohio State University.
Figure 1. Absorption-mode, magnitude-mode, and enhanced absorption-mode FT spectra of a time-domain signal consisting of three exponentially damped sinusoids of relative amplitude, 10:1:3. The frequency of the weakest signal is shown by an arrow. Note the improved (peak height)/(rms baseline noise) ratio for the enhanced absorption-mode display (see text).

Figure 2. Absorption-mode, magnitude-mode, and enhanced absorption-mode FT/ICR mass spectra of an experimental time-domain N$_2^+$ signal. The higher resolution of the enhanced absorption-mode compared to magnitude-mode is especially obvious.

References
ICR ORBITAL RADIUS DETERMINATION

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Measurement of the ion cyclotron orbital radius of an ion packet is of fundamental importance for several reasons. First, it provides for better quantification of ion abundances (from which ion-molecule reaction equilibrium constants are determined) from FT/ICR mass spectral peak areas. Second, the energy threshold for collision-induced (or collision-activated) dissociation, CID (or CAD), can be measured more accurately if the ICR radius (and therefore the ion translational energy) is known [1,2]. Third, the detection limit of an FT/ICR instrument may be determined directly by combining the measured ion packet cyclotron orbital radius with the measured ICR signal (for known receiver amplifier gain and equivalent capacitance of the entire detection circuit) to yield the number of ions which contribute to the signal [3].

Elsewhere, we showed that a standard cubic FT/ICR ion trap is capable of, but not ideally suited for, measuring ICR orbital radius [4-5] by measurement of the ratio of the magnitude of the third harmonic to that of the fundamental ICR signal. In this work, we present experimental results from a new six-detection electrode ion trap specifically designed for ICR radius determination (see Figure 1D). Because the new trap has four sets of detection electrodes, four different wiring configurations are possible (see Figure 1). This novel trap causes the signal to have a higher third harmonic content, thereby providing better sensitivity in the radius determination measurement. Theoretical values of the ratio of the third-to-first harmonic magnitudes are plotted in Figure 2a vs. ICR orbital radius, normalized to the maximum allowed radius. Note the increase in the sensitivity of this ratio, especially in configuration D. From these calculated values, the radius of the ion may be determined by measuring the relative magnitudes of the third and first harmonics. In Figure 2b, radii (normalized) determined in this way, for benzene molecular ion (on-resonance, single frequency excitation), are plotted versus the normalized post-excitation cyclotron radius predicted under the infinite electrode approximation for each of the four wiring configurations. Under the same ionization and excitation conditions, radii measured with the four different wiring configurations demonstrate good agreement with one another. Furthermore, in all four cases, the last measured radius before complete radial ejection is near the maximum allowed radius.

This method for determination of the ICR cyclotron radius should prove extremely useful for determining either the number of ions for a given signal or the energy of the ion packet before collisional dissociation. Fuller details will be presented later [6]. This work was supported by NSF (CHE-9021058) and The Ohio State University.

REFERENCES

Figure 1. Cross-sections through a novel ion trap designed for ICR orbital radius determination. Division of the detection plates into three isolated sections permits radius determination based on four different detection configurations. Experimentally determined radii from all four configurations are compared in Figure 2b. Configuration A is standard.

Figure 2. (a): Calculated ratio of the magnitude of the third harmonic to that of the fundamental vs. the normalized ICR orbital radius for the four different cell configurations of Figure 1. (b): Ion orbital radius obtained from measured values of the ratio (and the results of Figure 2a) versus the radius predicted for on-resonance excitation conditions and the infinite electrode approximation (see text). $V_{pp}$ is the peak-to-peak single-frequency rf excitation voltage; $T$ is the excitation period; $a/2$ is the maximum radius allowed by the trap, and $B$ is the applied magnetic field induction.
INTERFACING HPLC to FTMS USING THERMOSPRAY DEPOSITION ON A MOVING BELT

Mehdi Moini
Department of Chemistry and Biochemistry,
University of Texas at Austin, Austin, Texas 78712.

INTRODUCTION

Liquid chromatography/mass spectrometry (LC/MS) is an increasingly popular technique for the analysis of complex mixture not suitable for GC/MS with particular emphasis on polar, nonvolatile or high molecular weight compounds. Many biomedical analyses are performed by HPLC, which obviates inherent thermal lability and derivatization problems and thus may demand less complex sample preparation than GC. Among the several approaches to LC/MS coupling the moving belt is particularly suitable for HPLC/FTMS. This approach results in complete removal of the mobile phase between the LC column exit and the FTMS. This complete removal is important in terms of high pressure limit, space charge effect and chemical interferences contributed by mobile phase solvents in the analyzer cell of the FTMS.

APPARATUS

A device is described for coupling HPLC to FTMS using a variable speed, stepping motor driven moving belt constructed on a 3/4” diameter 38” long solid probe (standard probe diameter for the Nicolet FTMS-2000). This shaft has been grooved on the top and the bottom to accommodate a 0.0625 inch wide, 0.003 inch thick belt when the shaft is inserted in the seals, as shown in Figure 1. A thermospray nebulizer deposits materials eluting from the HPLC column on to the continuously moving endless belt. The effluent from the HPLC enters the vaporizer through the 0.0625 inch o.d., 0.004-0.005 inch i.d. stainless steel capillary tube which is embedded at one end into an aluminum block. The block is heated by two commercial 100 W cartridge heaters. A thermocouple is embedded in the cooper block to monitor the temperature of the vaporizer. The temperature of the vaporizer is controlled by a variable autotransformer. The moving belt is 0.0625 inch wide, 0.003 thick Nichrome ribbon and may be used as an endless loop or open strand. An endless belt is made by spot-welding the terminal 2-3 mm of the 2.5 m long ribbon. The overlapping sections of the belt are ground to keep the belt thickness approximately constant (0.003 inch).
The belt is driven by a controlled stepping motor that drives the main pulley. The belt carries these compounds through three tunnel seals in a two stage vacuum lock arrangement into the FTMS where they may be vaporized by a laser or a variety of other techniques. A return pulley is mounted at the tip of the probe. Two wires are also terminated at the tip of the probe and are available for heating or mounting a grid to control the velocity of the laser desorbed ions. A Busch vacuum pump with 1 1/2 HP motor and 20 CFM was used for the first stage of pumping and an Alcatel mechanical pump with 3/4 HP motor was used for the second stage of pumping.

**Deposition Efficiency.** Deposition efficiency from the HPLC to the belt via the thermospray is approximately 70% of the response to the same size sample placed directly on the belt. The main reasons for this percentage are, the belt width with respect to the thermospray spot size, the reflection of some of the particles at the belt surface as a result of their momentum, and evaporation at the nozzle tip.

**Pressure Study In the FTMS.** When one stage of pumping was used, the source and analyzer pressures were ca $10^{-6}$ and $10^{-7}$ torrs respectively. When two stages of pumping was used, after 1/2 hour, pressure in the analyzer side was the base pressure, i.e., $8 \times 10^{-9}$ torr while the source pressure was $9 \times 10^{-8}$ torr.

**Why the Moving Belt**

1) The moving belt is compatible with the FTMS. The main characteristics of the moving belt are that it allows the complete removal of the mobile phase and the achievement of the base pressure in the analyzer cell of the FTMS.

2) The moving belt is versatile. The whole HPLC to MS interface is built on a solid probe that can be inserted when needed.

3) The moving belt is suitable for use with several desorption and ionization modes, including laser desorption, FAB, SIMS, El, CI and MPI. It is also completely compatible with all nonvolatile analytes, regardless of their polarity, size, or molecular weight.

4) The moving belt is inexpensive. The cost of this interface with respect to the other alternative, i.e., an external ion source, is minimal.

**Future work**

1) Experimentation with this device using laser and SIMS.

2) Since stainless steel belt cost only a few cents per foot, the continuous endless belt can be easily converted to an open loop design which uses a disposable belt. The disposable belt will eliminate belt cleaning and associated memory effect.

**REFERENCES**

Ion Spectra from Two-Laser Infrared Multiple Photon Dissociation Experiments

Mitchell A. Cheeseman and John R. Eyler, Department of Chemistry, University of Florida, Gainesville, FL 32611-2046.

We have developed a two-laser approach for studying infrared multiple photon dissociation of gaseous ions which uncouples the (initial) resonant absorption of the first 1 or 2 IR photons from subsequent up-pumping through the quasicontinuum to the dissociation limit. Thus, a low-power tunable laser can be used for probing the resonant absorption spectrum, and a more powerful CO₂ laser (which does not have to be tunable) can then be used to drive the photodissociation, resulting in a "probe-pump" experiment. This two laser photodissociation process is shown schematically in Figure 1.

Experiments were performed on a home-built FTICR mass spectrometer, which utilized an Extrel FTMS 1000 data station and superconducting magnet of nominal 2T field strength. Our standard ICR cell was replaced with a White type cell which incorporated three spherical mirrors (one on one side and two on the other) as receive plates. Outputs from a cw CO₂ and a pulsed CO₂ laser were reflected into the vacuum chamber through two ZnSe windows on a three window flange. The probe laser beam was focussed using a gold-coated spherical mirror (125 cm focal length), reflected from the turning mirror attached to the cell, and made up to 24 passes through the White cell. Ions were produced by electron ionization, unwanted ions were then ejected with normal swept frequency ejections, and a delay period of 500 ms was inserted to allow collisional cooling and ion relaxation toward the center of the cell. Ions were then irradiated by multiple passes of the resonant "probe" laser beam and the pump laser in 2-laser experiments. Following this, product and reactant ions were detected by standard FTICR methods.

Photodissociation spectra, which can be related directly to ion vibrational spectra, have been obtained for a number of simple organic molecular ions which have absorption bands within the tuning range of the CO₂ laser. Figure 2 shows an example obtained without use of the White-type cell, but a double pass of two CO₂ lasers in the probe-pump arrangement. For comparison, in recent work infrared spectra of a number of organic ions have been obtained utilizing a single moderate power resonant laser and the White-type ICR cell. The resonant 'probe' laser was tuned to various wavelengths at constant power and mass spectra showing the extent of photodissociation were taken, thus providing an infrared spectrum of the ion under study. An example of such spectra is shown in Figure 3. Comparison of this technique with the standard double pass technique used to produce Fig. 2 shows a dramatic enhancement in photodissociation for all of the ions studied when using the multipass cell.

A difference frequency accessory for our Nd:YAG-pumped dye laser has been installed which will provide tunable output in the 3000 - 3600 cm⁻¹ wavenumber region. This laser will be used as the probe laser in the "probe-pump" scheme (with a CO₂ laser still used as the pump laser) to provide infrared spectra of ions in wavelength regions corresponding to N-H, O-H, and C-H stretches.


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Figure 1

[Diagram showing dissociation threshold, pump laser, vibrationally excited ion, and trapped ion.]

Figure 2

[Graph showing normalized signal against wavelength (μm) with peaks at 9.95, 10.05, 10.15, 10.25, 10.35, 10.45, 10.55 μm.]

Figure 3

Narrow Band Infrared Spectrum of Diglyme

[Graph showing wavelength (cm⁻¹) against intensity (arbitrary units) with peaks at 930, 935, 940, 945, 950, 955 cm⁻¹.]

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Using 4-nitroaniline as a matrix, we have successfully obtained the spectrum of melittin (Figure 1). The key to our success was in choosing a suitable matrix, such as 4-nitroaniline. Figure 2 contains the spectrum of renin substrate using the same matrix (4-nitroaniline). Based on our experience with different matrices such as 2-nitroaniline, 3-nitroaniline and 2,4-dinitroaniline we feel that a good matrix should have: 1) an electronic transition corresponding to the wavelength of the laser used (energy of photon), 2) higher acidity in its electronically excited state (of course, compounds with higher basicity in the excited state can be used for negative ion production), and 3) a resonant stabilized structure in its excited state after transferring the proton (a stable deprotonated matrix anion). These findings are in agreement with our proposed mechanism for matrix assisted laser desorption. In laser desorption experiments high mass ions are formed with appreciable kinetic energies (> thermal) and require higher trapping voltages for efficient trapping because conventional radio frequency excitation methods do not uniformly excite the ion ensemble.
Figure 1. Melittin

Figure 2. Renin substrate
A New Laser Microprobe/FTMS System
Based on an Electromagnet

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The IBM-Endicott Laser Microprobe/FTMS system\textsuperscript{1} has been used in many fundamental and applied studies in order to obtain qualitative elemental and molecular information on small spots and thin films of interest in electronics packaging applications. The system was designed around an Extrel FTMS/2000 Fourier Transform Mass Spectrometer with a 3.0 T superconducting solenoid and a 2 in. cubic cell. The performance of this system has been described previously.\textsuperscript{2}

This system has limitations for routine analysis of some types of samples. Due to the configuration of the sample lock and probe rod, the sample size is limited to 7/8 in. in diameter or about 1 cm. square. This is a serious size constraint, since it means that larger samples have to be cut up for analysis, effectively destroying them. The 2 in. cell, along with the restrictions of the bore of the vacuum chamber, limit the focal length of the objective lens to 60 mm or more. A sample motion system\textsuperscript{3} was designed for the probe rod, but it is difficult to make it compatible with the high magnetic field and the high vacuum. Some sample motion can be accomplished by moving the probe rod, but the motion is limited and the sample must be very accurately positioned. Finally, the visualization and illumination system was not very flexible. The illumination was limited to the direction perpendicular to the visualization optics, so shiny samples gave a reflection into the eyepiece but some other samples were completely dark or had no contrast. This problem makes it difficult to identify or locate small spots for analysis.

Therefore, a new laser microprobe/FTMS system was designed and built to overcome these limitations. Due to the geometry of the electromagnet pole caps, much more space is available perpendicular to the magnetic field than in the solenoid system. The Electromagnet Laser Microprobe (ELM) allows a sample size of 15X15 in. The system uses a 1 in. cubic cell, which allows room for a 25 mm focal length lens. An XYZ stage provides greater sample motion, and the system is designed for flexibility in positioning the optics.

The ELM system is suitable for a number of particular applications. It can be used to analyse contaminants on large substrates, boards, or panels without destroying them by cutting them up. It can be adapted to use with difficult samples, rather than requiring the samples to be adapted to the instrument. Since the samples remain in their original size, they can be processed subsequent to analysis, allowing the determination of changes caused by the later processing. Finally, the ELM should allow faster analysis of routine samples.

A detailed diagram of the vacuum system and optical system of the ELM is shown in Figure 1. The sample size of 15X15 in. is limited by the vacuum chamber in height and width. The maximum thickness is 1/2 in., which is sufficient for most electronic circuit boards. A sample lock provides rapid introduction of 1X4 in. samples. For larger samples, the end flange of the chamber can be removed for mounting and positioning the sample in the correct location. Since the cell and all the optics are mounted to the end flange, the sample can be positioned in air exactly as it will be located inside the chamber in vacuum. The cell is designed to be moved fairly readily to the portion of the sample which is of interest.

The sample is moved on a probe rod mounted on an XYZ translation stage bolted to the gate valve. The range of the translation stage is 1X1X2 in.
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The Z axis motion of the stage brings the sample into focus. Motion is accurate to .001 in. In the Y and Z directions, motion of the probe rod is limited by the gate valve, but in the X direction the motion is limited only by the chamber.

The sample visualization and laser focussing are both accomplished with the same fused silica, 25 mm focal length lens and Al mirror. The lens is mounted next to a hole in one excite plate. The viewing angle is about 60° from the sample normal, although the sample can be rotated to some extent to make a smaller angle. This optical design is fairly simple and is similar to the previous solenoid system.

The electromagnet for the ELM was purchased from GMW Assoc. The power supply was selected for its exceptional stability, and it was tested to give a drift of <6 ppm after warmup. Due to the stability, a Hall probe feedback sensor is not required. The pole caps are 10 in. in diameter, and the pole gap is variable from 0 to 6 in. For a 3 in. pole gap, the maximum field is 1.3 T.

The laser and interface systems have been described previously, and have only been slightly modified. A Continuum YG660 Nd:YAG laser operated on the fourth harmonic provides 266 nm laser radiation. The laser is triggered from an IBM PC-AT interface. The AT communicates with the Nicolet 1280 computer via an Rs232 port to begin experimental sequences. The communications also allows downloading of spectra or data to the AT for plotting or processing.

At present, the ELM system has not been tested due to problems with the vacuum system. Therefore, the ultimate spot size and mass resolution have not been determined. Further work on the system is necessary to determine the general utility of the system for routine sample analysis.

References

2. Extrel FTMS, Madison, WI.
3. GMW Associates, Redwood City, CA.
5. Continuum (formerly Quantel International), Santa Clara, CA.

Figure 1: Diagram of the Electromagnet Laser Microprobe/FTMS vacuum and optical systems. The diagram is to scale.
The ion chemistry of XeF₂ after electron impact ionisation is studied. The product ions XeF⁺, XeF₂⁺, XeF₂⁺, XeF₂⁺ and XeF₂⁺ are formed. Of special interest is XeF₂⁺, also known in the condensed phase. For this ion the following formation reactions are possible:

A: by addition: \( \text{XeF}^+ + \text{XeF}_2 \rightarrow \text{XeF}_2^+ \)

B: by fluoride transfer: \( \text{XeF}^+ + \text{XeF}_2 \rightarrow \text{XeF}_2^+ + \text{XeF}_2 \)

In the negative mode XeF₂⁻ ions are observed.

For the investigations we use a modified Spectrospin CMS 47 ICR Spectrometer. We operate at a magnetic field strength of 7.02 Tesla. The ICR cell is of 43 mm length and 34 mm diameter. The direct inlet system is made of stainless steel. The sample is stored in a small autoclave directly connected to the UHV system by a leakvalve. The small tube connecting autoclave and leakvalve can be pumped separately. The autoclave is filled with 100 mg XeF₂. The surface of the inlet system and the ultrahigh vacuum region are conditioned with SF₆ / F₂ to suppress surface reactions.

Reactions of XeF₂ at a pressure of 1.6 x 10⁻³ mbar are shown in the following diagram.

The curves show the ions of \(^{132}\text{Xe}^+\), \(^{131}\text{Xe}^+\) (m/z=161), \(^{130}\text{Xe}^+\) (m/z=320) and ten times amplified \(^{132}\text{Xe}^+\) (m/z=228) and \(^{130}\text{Xe}^+\) (m/z=301).

By ejection of a single or several isotops during the reaction delay it is possible to modify the pattern of an educt ion. The modified pattern of the selected educt shows an influence on the product peaks, only if the modified xenon isotope is transfered to the product ion. The following figure shows the calculated pattern of \(^{132}\text{XeF}_2^+\) after modification of \(^{132}\text{Xe}^+\) and \(^{129}\text{Xe}^+\) isotops.
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The next diagram shows the experimental data. The ions of XeF\(^+\) (m/z=161 and m/z=149) are ejected during the reaction time.

Changes in the isotopic pattern of Xe\(^+\) and XeF\(^+\) do not yield any alterations in the isotopic pattern of the ionic reaction products. This can only be explained by a reaction with a dimeric XeF\(_2\) (Xe\(_2\)F\(_4\)) (or higher oligomers). The observed reactions are dissociative fluoride-transfer:

1. \(\text{XeF}^+ + \text{Xe}_2\text{F}_4 \rightarrow \text{Xe}_2\text{F}_3^+ + [\text{Xe}^+, 2\text{F}]
2. \(\text{Xe}^+ + \text{Xe}_2\text{F}_4 \rightarrow \text{Xe}_2\text{F}_2^+ + [\text{Xe}^+, 2\text{F}]
3. \(\text{Xe}^+ + \text{Xe}_2\text{F}_4 \rightarrow \text{Xe}_2\text{F}_3^+ + [\text{Xe}^+, 3\text{F}]

In future we plan to investigate the reactions of XeF\(_2\) with the dioxygenyl cation, rare gases and halogens. We also want to analyse xenonoxo and halogen compounds. A pulsed inlet system has already been developed. First experiments with mixtures of xenon, xenon difluoride and carbon dioxide show formation of XeFCO\(_2\)\(^+\).

Work is granted by Deutsche Forschungsgemeinschaft. We thank EMG Bremen, especially Mr. K. H. Kaiser from EMG Bremen for support.
A high precision solid sample probe has been developed to analyse the ICR spectra of some polyperfluoroethanes like polytetrafluoroethylene, polyperfluoroethylenepropylene and polyperfluoroalkoxyteflon. We have also been able to obtain the ICR spectrum of the perfluorinated polyether KRYTOX 143 AZ (Dupont). A prototype Spectrospin FT-ICR spectrometer with a cylindrical ICR cell has been employed. The probe is introduced into the ultrahigh vacuum system via a differentially pumped vacuum lock. The pushrod used has a length of 900 mm and a diameter of 22.6 mm. The top of the pushrod is equipped with three electrical feedthroughs, one suited for a high voltage of 12 kV. The solid trapping plate of the ICR cell in front of the pushrod is replaced by a copper grid to increase the ion yield. The formation of negative ions from the solid sample is assisted by the Penning discharge induced by the magnetic field of 7 Tesla and the high voltage in the range from -3 to -8 kV which depends on the sample. A mass range up to 1200 is covered for the teflon derivatives and up to 1800 for the polyether. For each of the samples characteristic fragmentation patterns are obtained. However, it has not been possible to determine the molecular weight distribution of the polymers. Most of the mass spectra of teflon described in the literature are restricted to the lower mass range (m/e < 360)\(^1\). Recently some TOF mass spectra\(^2\) and Laser Desorption FT MS spectra\(^3\) have been described.

\[
\Delta (\text{CF}_2)_n^+ \quad n = 4 - 21
\]
Figure 1 shows the ICR spectrum of polytetrafluoroethylene at a pressure of \(2 \times 10^{-7}\) mbar. The spectrum is obtained at a high voltage of \(-8\) kV. A characteristic fragmentation pattern appears with a mass difference of 50 amu. This corresponds to ions of the formulas \((\text{CF}_2)_n^\text{-}\). The strong signals in the lower mass range belong to the ions \(\text{C}(\text{CF}_2)_n^\text{-}\).

The second example (Fig. 2) shows the ICR spectrum of polyperfluoroalkoxyteflon at a pressure of \(1 \times 10^{-7}\) mbar. The high voltage used is \(-5\) kV. The characteristic fragmentation pattern is \(\text{O}(\text{CF}_2)_n^\text{-}\) ions with formulas \((\text{CF}_2)_n^\text{-}\) and \(\text{CF}(\text{CF}_2)_n^\text{-}\) are also formed. Fragmentation products of low intensity that occur in both spectra are \(\text{C}_2^\text{F}(\text{CF}_2)_n^\text{-}\), \(\text{C}_2(\text{CF}_2)_n^\text{-}\) and \(\text{C}_2\text{F}(\text{CF}_2)_n^\text{-}\).

Finally a possible ionization mechanism will be discussed. At the surface of the counter electrode electrons are emitted due to the high voltage. These electrons are spread in all directions. If they hit the surface of the solid sample ions and neutrals will be sputtered from this surface. Neutrals that reach the space between pushrod and ICR cell will be ionized by electron attachment and detected inside the ICR cell. We call this discharge-assisted ionization.

Work granted by the Deutsche Forschungsgemeinschaft

3. EXTREL FTMS Application Report No. 6.
Simultaneous Trapping and Mass Spectrometry of Positive and Negative Ions in the ICR Cell

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The reactions of positive and negative ions in the gas phase are of great interest in astrophysics, atmospheric physics, heavy ion plasmas and in chemistry. The study of the reactions of positive and negative ions is very difficult and despite their great importance only a few investigations have been published.

In the ICR cell until now only positive or negative ions or electrons could be trapped, due to the trapping potential applied. To trap ions of both polarities two methods are proposed: (i) confinement by an additional RF electric quadrupole potential, or (ii) confinement by an additional static electrical potential. In this paper the second method and its experimental realization will be described.

The investigations are performed with a prototype Spectrospin FT ICR spectrometer CMS 47. It is equipped with a superconducting magnet of 7 T field strength, and controlled by an Aspect 3000 computer. The base pressure is in the $10^{-10}$ mbar range.

In Fig. 1 the cylindrical ICR cell employed for the study is shown. It is equipped with two additional grids in front of the trapping plates. The trapping plates and the grids are at opposite potentials, e.g. the grids at positive and the trapping at negative potentials of a few volts. With this arrangement positive and negative ions are trapped simultaneously. Excitation and detection of the ions of both polarities can be performed either simultaneously or after ejection of one kind of ions with the standard detection scheme, which employs the two cylinder segments $E$ for excitation and the two segments $D$ for detection (cf. Fig. 1).

The new method is applied to the ion chemistry of sulfur hexafluoride. Electron impact ionization of this compound yields as the major positive ions $\text{SF}_5^+$, $\text{SF}_4^+$, and $\text{SF}_3^+$. No positive molecular ion is formed. With the near-thermal electrons, which are formed in large amounts in the ICR cell the negative molecular ion, $\text{SF}_6^-$, is generated, and
electrons of higher energy, also $\text{SF}_5^-$ is formed. In the mass spectrum all the ions are detected. Their relative intensities depend on the grid and trapping plate voltages. At constant trapping plate voltage a range of grid voltages exist where both positive negative ions are trapped. The grid voltage can also be adjusted to operate the ICR cell in the "normal" mode to trap only one polarity of ions. Figure 2 shows a spectrum where the ions $\text{SF}_5^+$ and $\text{SF}_6^-$ dominate. The trapping potential was $-15$ V and the grid potential was $+0.6$ V.

With SIMION program (1) the trajectories of the trapped ions are calculated and the mechanism of the simultaneous trapping can be shown.

With the new method, the reactions of positive and negative ions can be studied with ICR, a new application of the method.

![Diagram](image1)

**Fig. 1.**

![Diagram](image2)

**Fig. 2.**

References


Work granted by the Deutsche Forschungsgemeinschaft.

The authors acknowledge help by Bruker Franzen Analytik GmbH, Bremen.
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Comparison of Detection Modes of ICR-MS for Intensity Accuracy
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Using an ICR-MS equipped with all three modes of detection - slow passage (SP), rapid scan (RS) and Fourier Transform (FT) - a comparison of ion abundance measurements has been made. SP detects only the absorption of energy, RS detects both absorption and emission and FT detects only emission. All three modes have been studied while varying experimental parameters, and compared for gas-phase basicity experiments.

The RS and SP hardware utilize a capacitance bridge detector, run by an IBM PC. The FT hardware involves a partial IonSpec detection system. For all three, interface boards on the PC generate the pulses and their timing. A 1.3 T electromagnet and 2.5 cm cubic cell were used.

Non-reacting Ions (CHC12⁺ Isotope Ratio):

The number of ions in the cell affects the apparent isotope ratio. The number of ions was assumed to be proportional to the product of the emission current and grid pulse duration. Better results are obtained with lower numbers of ions in the cell for all methods.

The magnitude of the detected signal should be linearly proportional to the excitation RF amplitude, up to ejection. For each method, the 85/83 ratio from the CHC12⁺ fragment from CHCl3. All 3 modes give slightly lower than theoretical values. The RS data are much less affected by changes in excitation RF amplitude than FT. (FT spectra were corrected by division by the excitation spectrum.)

Reacting Ions:
To determine if the 3 modes of detection yield similar results for a common thermochemical measurement made by ICR-MS, two basicity equilibria were studied:

\[ \text{CHC}_2\text{H}_4\text{OSO}_{2}^- + \text{H}_2\text{O} \rightarrow \text{CHC}_2\text{H}_4\text{OSO}_{2}^+ + \text{H}_2\text{O} \]  [12 dalton (12%) difference in masses]

\[ \text{C}_6\text{H}_5\text{BRH}^+ + \text{MeOH} \rightarrow \text{C}_6\text{H}_5\text{Br} + \text{MeOH}_2^+ \]  [124 dalton (37%) difference in masses]

For the first system, the measured ΔG for all 3 methods was within the ±0.1 kcal/mol error generally associated with the method.

<table>
<thead>
<tr>
<th>Mode</th>
<th>RF(mV)</th>
<th>ΔG (kcal/mol)</th>
</tr>
</thead>
<tbody>
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<td>SP</td>
<td>25</td>
<td>-0.88</td>
</tr>
<tr>
<td>RS</td>
<td>50</td>
<td>-0.85</td>
</tr>
<tr>
<td>RS</td>
<td>100</td>
<td>-0.82</td>
</tr>
<tr>
<td>RS</td>
<td>200</td>
<td>-0.89</td>
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<tr>
<td>RS</td>
<td>1500</td>
<td>-0.81</td>
</tr>
<tr>
<td>RS</td>
<td>5000</td>
<td>-0.81</td>
</tr>
</tbody>
</table>

For the second (wide mass range) system, the measured ΔG varied with detection mode: Literature data suggest ΔG = +0.5 kcal/mol.

<table>
<thead>
<tr>
<th>Mode</th>
<th>RF(mV)</th>
<th>ΔG (kcal/mol)</th>
</tr>
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<tbody>
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<td>RS</td>
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</tr>
<tr>
<td>RS</td>
<td>200</td>
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</tr>
<tr>
<td>157⁺/33⁺</td>
<td>0.7</td>
<td>-1.5</td>
</tr>
</tbody>
</table>

The unusual results obtained by RS are probably a function of the detection method. In this experiment, C6H5BRH⁺ was detected first (increasing frequency sweep). Before detection of MeOH2⁺ (ca. 100 msec), the excited C6H5BRH⁺ could react further with methanol and increase the MeOH2⁺ population. In an FT experiment all ions are excited and detected simultaneously and do not have time to react further.

We conclude that:
1. RS is less affected by experimental parameters (excitation RF amplitude, # of ions in the cell) than either FT or SP.
2. FT, RS and SP all give similar results for reacting ions over a narrow mass range.
3. Errors may be introduced in RS equilibrium experiments over wide mass range, while FT experiments are not affected. Sweep direction may alleviate the problem in RS experiments.

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A MECHANISM FOR POOR HIGH MASS PERFORMANCE IN FTMS.
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INTRODUCTION

The need for high mass capabilities has increased due to ionization techniques such as electrospray[1] and matrix assisted laser desorption.[2] Fourier transform mass spectrometry (FTMS) is a high mass, high resolution, multichannel analyzer which is being coupled to these techniques[3]. The potential of FTMS is seldom achieved with small ion populations such as those generated with large biomolecules. The reasons are likely to be electrical in nature. Scaling experiments show that elimination of peak splitting and optimization of resolution are achieved by an increase in ion number, or simple compensation of the trapping plates.[4] Russell et. al suggest that ion cloud expansion would result in loss of ion phase coherence and signal.[5] Expansion by long delays between ionization and detection, electrical effects, and ion partitioning or injection are proposed as possible mechanisms. Here we describe new experimental evidence that ion cloud expansion is a cause of signal loss. We propose and present evidence of a new mechanism for mass dependent cloud expansion.

THEORY

In anharmonic wells, coupling can occur between the radial and z modes, which leads to parametric resonance and radial expansion of the cloud. The masses that are affected are those whose radial frequency is an integer multiple of the z mode frequency in the trap center approximation. Thus these masses are given by \( m = m_e \left( \frac{1}{2k^2 + 1} \right) \).

EXPERIMENTAL

The FTMS system in this study consisted of a 2.54 cm cubic trap with split (ring and disc) trap plates[4]. Both plates are at 1 V in the uncompensated mode, and the ring is increased to 1.7 V in the compensated mode. A Varian V-3400 iron magnet with a V-FR250 Fieldial current regulator and a maximum field of 1.2 Tesla was employed. Except where noted, ion populations were from benzene at 2x10^-6 torr, ionized at 15 eV and 300 nAmps. Excitation was by a 0.35 Vb-n constant frequency r.f. pulse to each excitation plate. Detection was in the heterodyne mode using 4k data points. The behavior of various high mass ions at 7 Tesla were investigated by benzene ions by scaling the magnetic field, and time parameters appropriately.[4]

DISCUSSION

Signal loss due to cloud expansion is established by overt expansion of a methane ion cloud at 1.2 Tesla. This is achieved by application of a 1.6125 MHz r.f. burst on the D.C. bias of the trap plates. Methane is observed to mimic high mass behavior with respect to signal and ion number. The role of electric effects at high mass is established by 20,000 amu ion behavior modeled with benzene. Signal degrades as a delay time as long as ten seconds between ionization and detection expands the cloud. Compensation moderates the electric effects, and there is no loss in signal within the delay period.

Trap resonance is a mass dependent, radial expansion mechanism and apparently similar to those observed in magnetic bottles.[6] The figure shows the mass range of a 2.54 cm cubic trap in a 7 Tesla field as modeled with benzene. Regions of signal loss are centered at approximately 14,500, 34,500, and 91,000. Not shown here are calculation and experimental results which demonstrates that compensation shifts the resonance centered at 91,500 to a lower mass. Effects of compensation on the less intense resonances are under investigation.

Anharmonic traps should produce more extensive coupling between modes and produce wider and stronger resonances. We propose that harmonic wells are the appropriate direction for trap designs.

REFERENCES

1. Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. Science 1989, 246, 64.
The mass range of a 2.54 cm. cubic trap in a 7 Tesla field as modeled with the benzene ion. The legend indicates beam times. Trap resonance is a mass dependent cloud expansion mechanism, which causes regions of signal loss centered at approximately 14,500, 24,500, and 91,500 amu.
An Improved Pulsed Cold Trap/Pulsed Valve Interface for Trace FTMS Analysis.

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INTRODUCTION

For the analysis of volatile compounds, high resolution gas chromatography (HRGC) is far superior in separation characteristics to any other method. In terms of chemical analysis, Fourier transform mass spectrometry (FTMS) has been shown to be superior to sector instruments in the areas of ultra-high resolution, multi-channel detection, and fast scan capabilities\(^1\). With this understanding, the coupling of HRGC and FTMS should yield an analytical system capable of trace analysis for most volatile chemical species.

HRGC/FTMS in a single cell instrument is performed most efficiently via the use of a high speed pulsed valve\(^2\). Due to the narrow pulse width of the sample, with respect to the eluting time of the GC peak, we calculate that roughly 1% of the peak is actually detected while 99% is pumped away. If the entire GC peak were trapped, concentrated, and directed into the cell in a single pulse, the limits of detection should improve by approximately 100 times.

OBJECTIVE

To actually detect 100% of an eluting analyte, some basic requirements for a perfect cold trap interface must first be satisfied:

- The temperature gradients of the cold trap must be narrow and very well defined
- The width of the sample plug eluting from the cold trap must be very narrow in time (<20 ms)
- The cold trap interface must be capable of introduction into the vacuum system in close proximity to the FTMS cell to alleviate any diffusion broadening of the analyte plug

COLD TRAP DESIGN

The cold trap region is established and defined by thermal contact between a section of 0.635 mm o.d. x 0.305 mm i.d. stainless steel tubing brazed between two pieces of 6.35 mm brass plates. To one end of this tubing is attached the GC column while the other end is connected to the sample inlet of the pulsed valve. Channels have been milled in the brass stock to allow for the flow of liquid nitrogen. In this manner, the trap reaches near liquid nitrogen temperatures, while the thickness of the brass defines the width of the trap.

To control the temperature gradients of the cold trap, a set of moveable heater blocks have been positioned on either side of the trap at a separation of 0.305 mm. This forms a gradient of approximately 300°C over a distance of 0.305 mm.

To pulse the analyte from the trap we must invert the temperature of the cold trap. This is accomplished by passing a closely controlled current across the cold brass plates to heat resistively the steel tubing across its width. The variable amperage is supplied by a custom built power supply under computer control (SEE FIGURE 1).

As the analyte is vaporized and re-entrained into the helium flow of the carrier gas, a computer controlled pulsed valve opens for sample admission. By varying individual parameters, values for each interface sequence step can be optimized.

COLD TRAP EVALUATION

By passing a current of varying amperage across the width of the cold trap we detect gross temperature changes of 430°C for 850 and 700 amps at current pulse widths of 10 and 15 ms respectively. Under liquid nitrogen cooling and a current of 850 amps for 10 ms, we are able to invert the trap temperature from -140°C to +20°C at the center of the trap, and from -90°C to +180°C at the edge of the trap (SEE FIGURE 2).

Experiments show that the efficiency of the trap at producing narrow pulse widths is dependent on the amount of analyte sampled (SEE FIGURE 3). This phenomena is possibly due
to saturation of the trap. This reinforces the claim that this technique is for trace analysis.

The capability of our trap in creating very narrow pressure profile widths becomes evident in FIGURE 4. From this graph it is apparent that there is a problem due to dead volumes. By introducing an auxiliary helium flow at various pressures we can sweep out this volume to arrive at a profile width which is indicative of the cold trap.

Our future work will attempt to produce a better pulsed valve resulting in a substantially lower dead volume, and hopefully result in the ability to locate the trap in close proximity to the FTMS cell.

REFERENCES
Ions in an FT/MS® cell can be trapped, excited for detection, excited for collisional activation, or excited for ejection. Stored waveform inverse Fourier transform (SWIFT™) excitation is a technique for selectively coupling these characteristics over a range of ions, or in other words, "tailoring" the ion excitation energy profile in the mass domain. The advent of memory-based waveform generation for ion excitation allows complex pre-experiment excite waveform tailoring, which leads to analysis time savings and more effective experiments. Traditionally FT/MS has been calculation intensive after the experiment. By budgeting more time before an experiment, that time may be made up later by less stringent post-processing, and the high-resolution, user-friendly features of FT/MS can be applied to a wider range of problems.

The excitation and detection parameters in FT/MS are equivalent. The length of time spent in excitation affects the ultimate ion selectivity in the same way that the length of time in detection affects mass resolving power. In the ultimate sense, selectivity and resolution are constrained by pressure-limited collisions of cyclotroning ions. Historically, only the detection parameters have extended into the pressure-limited collision constraint. Recent developments in SWIFT hardware allow broadband, ultrahigh-resolution mass-selected excitation. This feature has been implemented in a versatile, user-friendly FT/MS system.

Ultrahigh-resolution detection is improved by using ejection sequences to remove interfering ions from the cell. We proposed that the same technique would improve excite resolution. Accomplishing ion ejections with a sine wave or swept sine wave (chirp) has its limitations. The swept sine wave typically induces an offset and side-lobe energy to ion species remaining in the cell. Successive swept sine wave ion ejections have been limited to low-resolution because of the accumulation of these effects. Combinations of low- and high-resolution chirp ejections improve the situation somewhat; however, SWIFT techniques combine multiple-band excitation in to a single, apodized, and offset-corrected event. We show how SWIFT can be used to 1) improved resolution, 2) provide parent ion selectivity for MS/MS experiments, and 3) provide ultrahigh selective excitation.

All results were run on a standard EXTREL FTMS instrument with a SWIFT/Cell Controller module. The system has a dual ion source/analyzer with a 3-Tesla magnet. For collision-induced dissociation (CID), argon was introduced through a static valve on the analyzer side. Some of the features of the SWIFT module used in these experiments include:

- 65.5 kHz to 8 MHz (Nyquist) memory-based waveform generator with 1 megaword of 12-bit data.
- 8 MHz (Nyquist) swept frequency generator with ultrahigh-resolution (heterodyne) capabilities.
- Data system programmable filters and attenuators, and high-speed switching analog electronics.
- Unified, user-friendly software package.

1) Figure 1A is ultrahigh-resolution detection and isolation of Bromochlorobenzene isotopic ions at m/z 192 by using a single SWIFT excitation for isolation.

2) A mixture of anisaldehyde and d-limonene is a system that requires more than a resolving power of 2,000 for isolation. Once isolated, low-resolution detection can be used with their CID spectrum to identify each component. In order to selectively eject each of the molecular ions, a SWIFT waveform was produced. It is very easy to switch between the two samples by changing only the SWIFT reference by the difference in their masses. Figure 2A and 2C show the ion isolations, and Figure 2B and 2D show their respective CID spectrum.

3) An ultrahigh-resolution SWIFT excitation was used as the excite-for-detection with a notch having resolving power of 215,000, which leaves a non-excited millimass unit wide ion species in the mass domain.
the center of the cell (Figure 3A). By successively scanning, and incrementing the SWIFT reference mass over the m/z 192 region, the following isolations were achieved (Figures 3B, 4 - 7). The increment between each Figure in the series is 0.4 Hz which is equal to 0.3 millimass unit at m/z 191.

In conclusion, SWIFT techniques add to the spectrometrist's capabilities by affording high-resolution excitation capabilities, predictable ion radii and energies, apodized balanced waveforms, and reduced off-resonant effects. Tailoring excitation through preprocessing provides a versatile experimental platform, which is easy to use, simple to verify, and provides rapid isolation and analysis on ion species of very close mass-to-charge ratios.

Future enhancements to SWIFT preprocessing algorithms will undoubtedly speed calculation times, and provide more flexibility in the definition of “don't care” regions of the mass spectrum. This indicates an opportunity to develop algorithms for more efficient excitation and higher resolution selection.
Effects of Tailored Excitation Waveform Sampling Rate on the Signal Experienced by Ions In FTMS

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Stored waveform inverse Fourier transform (SWIFT) excitation is becoming a widely used technique in Fourier transform mass spectrometry (FTMS). SWIFT excitation involves the transmission of a signal (excite waveform) through the digital-to-analog conversion (DAC) of a stored waveform. The effects of the sampling rate of a digital waveform on the analog waveform experienced by ions in the ion trap is discussed.

It has been shown that oversampling of time domain signals in analog-to-digital conversion provides little improvement to FTMS spectra. For tailored waveform excitation, the sampling rate of the waveform customarily used is equal or slightly greater than the Nyquist limit of an excite band. However, this rate results in two artifacts in the power spectrum that ions experience during excitation, namely a roll-off of power in the excite waveform from low frequency to high frequency and large amplitude harmonics of the excite band. Depending on the sampling rate and the bandwidth of the excite waveform, the roll-off can be greater than 25% of the magnitude of the low-frequency edge of the band. The magnitude of the harmonics can be as high as 50% of the low-frequency edge of the requested excite band, as shown in Figures 1.

The roll-off and the harmonics are artifacts of DAC conversion. During the excitation, a zero order recovering filter is normally used; that is, the electronics step through a digital waveform point-by-point and the value of each data point is held for the length of a dwell time period of the excite waveform. Discrete mathematics predicts that there are artifacts in the frequency domain response of the waveform.

The DAC of a waveform by a zero order recovering filter is a process of convoluting a filter box function on the SWIFT waveform. According to the Fourier convolution theorem, the frequency spectrum of the convoluted waveform is the product of a sinc function and the normal SWIFT waveform spectrum, as illustrated in Figure 2. Note that the normal spectrum of a discrete waveform is a periodic function with a period of $2f_n$, where $f_n$ is the Nyquist frequency of the waveform. The above phenomenon can also be explained qualitatively. The resulting analog waveform from DAC is a square-cornered step function. It is well known that square corners in the time domain is the sum of a series of harmonic frequency components. In addition, the step function analog waveform can be viewed as a sum of a series of waveforms with a fixed time delay between each other. From the Fourier transform shift theorem, this can also be translated as a series of waveforms with phase differences between each other that are applied simultaneously to excite the ions. The phase difference between the series of waveforms is proportional to the frequency and is within pi/2 radian; the lower the frequency, the smaller the phase difference between the series of waveforms. Therefore the low-frequency section of the spectrum has higher amplitude, resulting in a roll-off of power in the excite band from low frequency to high frequency.

When a first order recovering filter is used (by using the first derivative to interpolate the points in between the existing data points), improvement is seen in reducing the amplitude of harmonics, but the roll-off is more severe, as shown in Figure 1c. The same effect can be achieved with a low pass filter, as shown in Figure 1d. A second order filter was also tested; the reduction of harmonics is about the same as with first order recovering filter, but the roll-off is more severe than that with first order filter. Broadening the sinc function envelop will result in less roll-off. This can be achieved by reducing the width of the recovering window. One way to reduce its width is to shorten the holding period of data points during DAC. But this requires a higher voltage excite amplifier to achieve the same excite level. In addition, the harmonics have a higher amplitude. An electronic device may also be used to compensate the roll-off, but the closely spaced first harmonics may not be completely filtered out and the cutoff filter may also introduce roll-off. A purposefully predistorted waveform may also be constructed to produce a waveform with the roll-off compensated by dividing the original requested excite spectrum by the sinc function of the recovering filter. The simplest method is to construct the waveform at the sampling rate higher than the Nyquist limit, at the expense of requiring more computer memory and computational time. Computer simulation shows that when four times the Nyquist rate is used, the distortion of the spectrum of the waveform is within a few percent. Figure 3 shows the spectrum of a waveform generated at four times of the normal sampling rate.

In conclusion, a waveform free of roll-off and harmonic artifacts can be obtained when four times of the Nyquist sampling rate is used. Other methods also correct the DAC artifacts to some extent, but they have limitations. Balancing the trade-off of memory requirement and computational time, a better solution is to use twice of the Nyquist sampling rate, predistort the requested excite spectrum by the recovering filter sinc function and use an effective harmonic cut-off filter.
Figure 1.

a) Excite spectrum of a SWIFT waveform
b) Excite spectrum of the waveform through a zero order digital recovering filter.
c) Excite spectrum of the waveform through a first order digital recovering filter.
d) The spectrum of a SWIFT waveform detected by the detection electronics. A low pass filter was used to cut off the harmonics.

Figure 2. Illustration of the convolution of a waveform by a zero order recovering filter.

Figure 3.

a) The spectrum of a SWIFT waveform generated at four times the normal sampling rate detected by detection electronics. The detection sampling rate is twice that of the stored waveform.
b) Same as in a), except a low-pass filter was used to cut off the harmonics.

SECONDARY NONLINEAR RESONANCES
DUE TO EXCITATION ELECTRIC FIELD INHOMOGENEITIES
IN A CUBIC FT/ICR CELL

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In this study, we investigate the effects of excitation at frequencies away from \( w_+ \) and \( w_- \) due to electric field inhomogeneities from a sinusoidal excitation across the x-plates of a cubic FT/ICR cell [1].

To obtain analytically manageable expressions we first expand the exact electric potentials about the cell center in a Taylor series keeping terms up to the leading excitation inhomogeneities and quadrupolar trapping. Next we transform the cartesian equations of motion to a coordinate system co-moving with the linear quadrupolar system by the method of variation of parameters [2]. These coordinates correspond to the cyclotron, magnetron and z-mode amplitudes and their respective phases. In this amplitude/phase representation, the first-order resonance structure due to the excitation field inhomogeneities appears as terms with slowly-varying phases. The complete set of equations in amplitude-phase coordinates is still too complicated to be solved analytically. However, since the inhomogeneities are small perturbations and the resonances are usually widely separated, we obtain greatly simplified but still accurate expressions by averaging.

Two different classes of resonances are found: resonances dependent only on nonlinear terms and resonances which depend also on the linear excitation term. The second class of resonances is generally much weaker than the first. For the first class, secondary resonances occur at excitation frequencies near \( 2w_\pm w_+ \), \( w_+ \pm 2w_\pm \), \( 2w_\pm w_- \), \( w_\pm 2w_\pm \), \( 3w_\pm \) and \( 3w_\mp \). For the second class, resonances occur at \( (w_\pm w_-)/2 \), \( w_\pm /3 \), \( w_- /3 \) and \( w_\pm \). The first class includes the well-known z-mode coupling with the radial modes as well as coupling between the cyclotron and magnetron modes and sub-harmonic resonances of order 1/3.

Inspection of the coupling coefficients in the amplitude-phase equations of motion show that the resonances at \( 2w_\pm w_- \) and \( w_\pm \pm 2w_\pm \) have generally the strongest effect on ion motion.

A simple analysis of the resulting equations of motion, assuming each resonance is widely separated in frequency from all other resonances, shows that for each mode-coupling case there is one resonance which gives bounded trajectories while the other can give unstable motion due to the presence of invariants coupling the mode-amplitudes in the averaged equations. For example, the equations of motion for the resonance at \( w_+ + 2w_\pm \) possesses the constant of motion

\[ c = 2(w_\pm - w_-)a_\mp^z - w_\pm a_\mp \]

while for the \( w_+ - 2w_\pm \) resonance the constant of motion is

\[ c = 2(w_\pm - w_\pm) a_\pm^\mp + w_\pm a_\pm \]

where \( a_1 \) and \( a_3 \) are the cyclotron radius and z-mode amplitude, respectively. These two resonances have previously been predicted [3] and the above constants of motion can account for their observed behavior. The first of these equations is
a hyperbola while the second is an ellipse describing, respectively, unstable and stable motion. Additionally, for the averaged equations another invariant exists identifiable as the energy integral in a rotating coordinate system which along with the above constant of motion \( c \) can be used to reduce the original set of four first-order time-dependent equations of motion to a single time-independent equation integrable by quadrature. The averaged equations in the amplitude-phase representation contain just two modes at most. The mode-amplitude and phase of the third mode not in the averaged equations are constants of motion.

For the strongest secondary resonances we can take the ratio of the largest coupling coefficients in the averaged equations to the linear on-resonance cyclotron excitation expression

\[
\frac{\frac{d}{dt}}{d/B} = E/B
\]

To obtain the maximum order of magnitude of these terms. We find that the maximum value

\[
\frac{\gamma^2(E/B)}{d w_c} = 1.6 a_1 a_2 w_c
\]

where \( d \) is the cell length. If we take the case \( a_1 = a_2 = d/10 \) and \( w_c/w_c = 100 \), this ratio is on the order of 1, indicating that for large initial mode amplitudes, ejection is very rapid for the unstable \( z \)-dependent resonances. By minimizing either \( a_1 \) or \( a_2 \), the relative effect of this secondary nonlinear behavior can be reduced greatly. These secondary effects are probably minimal for most experiments as the initial mode amplitudes are usually much less than \( d/10 \).

However, in studies requiring extreme accuracy in ion abundance measurements, care must be taken when exciting ions at these secondary resonance frequencies because ion ejection and/or mode interchange could lead to anomalous results. If possible, a narrow pulse at the cyclotron frequency should be used rather than a sweep across many decades of frequency. This will minimize the excitation at the secondary resonances. For studies involving more than one mass (the vast majority of all studies), minimizing the frequency range of the excitation pulse is suggested to help prevent inadvertent secondary nonlinear effects.

A Fourier Transform Ion Cyclotron Resonance (FT-ICR) laser microprobe system, designed for the analysis of specific mineral grains in geological samples, was developed. Design criteria included the accommodation of standard geological thin sections (27 x 46 mm), a laser spot size of ca. 30 μm, sample placement to ca. 10 μm, movement of the sample of at least ±1/2" in the xy plane, sample viewing capability, and the ability to interface with a modified Nicolet/Extrel FTMS-2000.

The commercial solids probe that came with the system was limited in size to sample disks 5/8" in diameter and limited in sample placement to rotation about the z-axis. For the analysis of geological samples, an xyz manipulator was used to allow both larger samples and much more flexibility in sample positioning. A load-lock system was designed to allow the transfer of the sample onto the xyz manipulator under high vacuum, thus allowing manipulation of the sample under ultra high vacuum. The sample holder, load lock system, and xyz manipulator accommodates either standard 1" diameter or 27 x 46 mm geological thin section slides. With a resolution and repeatability of 5 μm along the x and y axes, excellent placement of the sample is accomplished. With the laser beam and other optics fixed, movement of the manipulator in the xy plane selects the target area for the laser while movement of the sample manipulator along the z axis provides for focussing of the laser on the sample surface. Movement in the xy plane allows not only positioning of a mineral of interest into the path of the laser for analysis but also allows a series of spectra to be taken across a particular area of interest (the resolution in this case is limited by the spot size of the laser). The ±1" movement of the xyz manipulator allows the full area of either size thin section to be analyzed.

The optical system includes a straight optical path inside the vacuum system along the z-axis to the sample through holes in the two trap plates of the FT/ICR analyzer cell. A 75 mm focal length planoconvex lens just outside the trap plate opposite the xyz manipulator serves both to focus the laser light onto the sample and to provide viewing of the sample before the analysis. A HeNe laser beam, collinear with the Nd:YAG laser shows the point where the laser beam is focussed, so choosing a particular mineral grain (or crystal zone within a mineral) on the sample for
analysis is simple. All of the alignment optics are outside the vacuum chamber, mounted on an optical table for ease in adjustment. For laser desorption, the Nd:YAG, operating at the fundamental wavelength of 1064 nm, is focused through the analyzer cell onto the sample just outside the opposite trap plate, producing ions directly from the solid sample, followed by subsequent ion excitation and detection with the FT/ICR electronics. Because a complete mass spectrum can be obtained from a single laser shot, subsequent laser shots can either probe deeper into the sample if the sample is not moved or spatial information about species present in the sample can be obtained if the sample is moved after each laser pulse.

A laser spot size of approximately 100 μm was attained by the optical setup before the addition of a 3x beam expander. The beam expander should reduce the spot size by a factor of 3, allowing more spatial information to be obtained from a geological sample.

Limitations and difficulties of the system include difficulty in performing electron impact experiments, a longer pumpdown time, viewing optics that are not so flexible, and fairly slow and tedious sample introduction. Electron impact experiments currently require the complete removal of the xyz manipulator and the focusing lens because they are both along the z axis. Future plans include the design of a filament that will mount on the end of the xyz manipulator and a shutter that will block the path to the focusing lens, preventing damage by electrons. The flexibility in sample movement and the ease in alignment of the optics more than makes up for the longer pumpdown time required for analysis. The 3" focal length lens for focusing the laser pulse and magnification of the sample image limit the resolution of the viewing system. A system with different paths for sample viewing and the laser beam is currently under consideration. Motorization of the xyz manipulator would provide greater resolution and repeatability and faster operation. As the xyz manipulator has the ability for field fitting of stepper motors, this would be a simple upgrade.

In summary, the system allows the laser microprobe analysis of individual mineral grains directly from standard geological samples. Information on the spatial distributions of chemical species can thereby be obtained that is not available by more traditional techniques.

As this system has flexibility in sample introduction, sample movement, and the optical system, a similar system could be readily used in other applications where mass spectral spatial information of solid samples is desired.
Spontaneous Coherent Magnetron Motion. Ion motion within the trapped ion cell may be described as the superposition of the trapping, cyclotron, and magnetron motions. Magnetron motion results from a combination of radial electrostatic forces and the magnetic forces and amounts to a drift of the center of cyclotron gyration along the closed isopotential contours of the radial electric field. Unlike the trapping and cyclotron motions, the amplitude of the magnetron motion will increase as the energy associated with this mode decreases. For FTICR experiments in which a well-behaved ion population is created along the cell axis, magnetron motion and the associated effects are minimized. However, for experiments in which ionization conditions are more extreme or not as well controlled, the magnetron effects may be significant. Moreover, under certain conditions, coherent magnetron motion may spontaneously occur, as evidenced by the direct observation of magnetron transients in the absence of an excitation event. Such motion has important implications for FTMS detection.

Examples of the coherent motion resulting in magnetron transients display an initial growth and subsequent decay in signal amplitude. The behavior shown corresponds to an increase in magnetron radius until ions begin to collide with the cell perimeter and are lost. Figure 1 is an expanded segment of a typical magnetron transient acquired following an LDI event in the cubic cell. The sinusoidal nature of the detected magnetron signal is evident as well as the high signal to noise ratio which implies a large magnetron radius and a high degree of coherence to the ion cloud.

Another feature of the magnetron transient is an evolution of the magnetron frequency with time. The initial frequency is higher than predicted based on the radial component of the trapping field alone, but quickly decays to the expected value. The initial frequency enhancement is attributed to space-charge effects which decrease as the ion cloud evolves. Evidence that space charge is responsible for the frequency enhancement is presented in Fig. 2, where a suspended-trapping event is applied to reduce the ion population and thus the space charge contribution to the radial electric field in the trapped-ion cell. The initial magnetron frequency is 950 Hz, 50% higher than the predicted value of 634 Hz. Following a brief suspended trapping event to eject some ions, the observed magnetron frequency is 670 Hz, within 6% of the expected value. This additional radial electric field component depends linearly upon the total number of ions (represented by ionization time). A linear shift in magnetron frequency is displayed until it levels off at longer beam times as the space charge capacity of the trapped-ion cell is achieved. The space charge in combination with the resulting image charge on the cell electrodes creates a net electric dipole.

The magnetron frequency varies with trapping potential because the radial electric fields responsible for magnetron motion are a component of the applied trapping potentials. For the cubic cell, this frequency shift can be predicted based on a quadrupolar approximation of the cell electric fields. Attempts to predict behavior for elongated cells may be unsuccessful if ion motion is not constrained to a region very near the center of the cell. Fig. 3 demonstrates the failure of the quadrupole approximation to predict the relationship between trapping potential and magnetron frequency for the elongated cell. From SIMION work, the cubic cell radial electric field is found to vary by less than 20% over a z-axis range covering 70% of the z-dimension of the cell, so the quadrupole approximation closely approaches the observed result for the cubic cell. In contrast, a variation in excess of 600% for the radial electric field exists over a 70% fraction of the elongated cell length. A prediction of 52.5 Hz/V is made for the slope of Fig. 3 using the average radial electric field over the full z-axis dimension of the cell. The behavior of ions confined to a smaller range of the z-axis motion approaches the predicted value because the quadrupolar approximation is valid at the center of the cell.

Resistive-wall Destabilization. Resistive-wall destabilization is a collision-independent ion transport mechanism which results from the destabilization of coherent ion magnetron motion in the trapped ion cell. This results in radial ion transport and potential ion loss for ion populations that exhibit a significant degree of coherent magnetron motion. This transport mechanism is an important factor to be considered in the experiment design process. Comparison of magnetron transients acquired with differing values of external circuit resistance provides a direct demonstration of the resistive-wall effect on magnetron motion. Fig. 4a and 4b demonstrate
growth proportional to the external resistance in the detection circuit of magnetron transients with time. In Fig. 4b, the eventual decay of the magnetron transient corresponds to the loss of ions at the cell perimeter and is direct evidence that resistive-wall destabilization is an important ion loss mechanism.

Coupling ion motion to a resistive external circuit is an effective means of removing energy from ions confined in an electromagnetic trap because the ion energy is dissipated by Ohmic power loss in the external resistance. Eq. 1 was derived to show that the growth of the magnetron radius due to resistive heating of the external circuit increases linearly with number of ions (approximated by ionization time), trapping potential, and resistance.

$$r = r_0 \exp \left( \frac{t}{\kappa} \right)$$

The behavior of magnetron motion is consistent with a radial displacement of the ion cloud. This displacement can occur as a result of the acquisition of an off-center ion cloud, or as a result of the resistive-wall destabilization of an initially centered cloud. If the external resistances associated with each electrode are not balanced, the asymmetrically distributed image charges induce a net force on the cloud which causes radial displacement.

Figure 1

![Figure 1](image1.png)

Figure 2

![Figure 2](image2.png)

Figure 3

![Figure 3](image3.png)

Figure 4

![Figure 4](image4.png)
Different from commercial FTMS data systems and data systems constructed by other laboratories, this simple modular data system for selective waveform excitation and programmable trapping experiments employ readily available, inexpensive "off-the-shelf" components. No hardware construction and minimal programming skills are required. System complexity is dramatically reduced with an arbitrary function generator (AFG) that drives, with appropriate amplification, all forms of radial ion excitation and z-axis trapping and excitation. Examples to be presented include SWIFT and multiple frequency excitation as well as the use of adiabatic compression of trapping potentials to drive ions to the center of the cell.

The spectrometer of the FTMS includes a 2.0T superconducting magnet and is pumped to pressures of $10^{-9}$ torr. The system is configured as an external ion source tandem time-of-flight FTMS. However, for this work an axial probe-mounted electron gun was installed to permit conventional electron ionization in a 5x5x10 cm trapped ion cell.

A block diagram of the data system is presented in Figure 1. The primary components of the system are a personal computer (PC), a dual channel arbitrary function generator, and a digital storage oscilloscope (DSO).

A typical pulse sequence for an FTMS experiment includes ion-quench, ion formation, ion excitation and signal acquisition events. The modular data system analog to this sequence is executed in the form of waveforms and triggers, as shown in figure 2. These waveforms are first created on the PC using W.A.V.E. and then downloaded and stored in the AFG memory for output when the experiment is initiated.

A useful feature of the DSO is the ability to perform independent signal averaging and Fourier transforms. The signal averaging feature reduces the demand for data transfers to the PC and frees the PC for other work during lengthy periods of data acquisition.

The simplest excitation waveform is the single frequency sine wave. This waveform is useful when it is desirable to observe or eject selected ions of a single mass-to-charge ratio. Multiple frequency waveforms are constructed by adding together any desired number of single frequency waveforms. As will be shown, multiple frequency excites are advantageous for quantitative observation or ejection of small numbers of selected ions. This type of excitation can be an attractive alternative to SWIFT for high resolution selective excitation measurements. If the excitation of a large number of ion masses or a range of masses is desired a swept frequency excitation waveform may be employed. In this case the waveform is still sinusoidal but the frequency is varied linearly with time. Such waveforms are useful for observing or ejecting a broad mass range of ions with a single waveform. However, it is not generally possible to effect both observation and ejection of different ions with a single sweep frequency excitation.

The technique of multiple frequency excitation was employed for simultaneous excitation of multiple selected ions, it offers selectivity equal to or better than the corresponding SWIFT excitation but is computationally much simpler to generate. Of interest here are significant advantages for detecting daughter ions resulting from a CID experiment. For example, because daughter ions are formed in regions of the trapped-ion cell where electric fields are less homogeneous, they should exhibit greater coupling between the cyclotron, magnetron, and trapping motions. Selective excitation is much less likely to excite magnetron and trapping motion than broadband excitation and thus should provide a more homogeneous cyclotron excitation. The stored waveform inverse Fourier transform (SWIFT) method can be used to effect simultaneous observation and ejections over any desired mass range with very high resolution. Thus, the technique combines the advantages of both discrete and swept frequency excitation. The desired waveform is initially described in the frequency domain and then an inverse Fourier transform is applied to create a time domain waveform. Quadratic phase encoding is employed to keep the dynamic range of the resulting waveform within the limits of the excitation amplifier.

The second output channel of AFG can generate versatile trapping and excitation waveform for application to the trap plates. This opportunity to manipulate trap plate potentials
of the ions. Adiabatic potential ramps have also been suggested as a means to accomplish axial ion compression and thus minimize temporal variation in image current signal strength due to relaxation effects. Programmed potential changes have been further employed in dynamic trapping experiments where they are used to control the mass range of ions collected from an external ion source. A more complex manipulation of trap potentials may be employed to deliberately excite the trapping motion of ions by superimposing a waveform of the appropriate frequency on the trap potential. Shown in Figure 3 are spectra to illustrate excitation of adiabatic potential ramps with the data system. Figure 3a is the output second channel, Figure 3b is FTMS detection at 0.5 V with severe axial ejection of low mass PFTBA ions, and Figure 3c is the result from execution of the trapping profile in Figure 3a to compress ions to the middle of the cell with a ramp to 3 V before detection at 0.5 V.

Figure 1. Block diagram of data system

Figure 2. Data system pulse sequence output by AFG.

Figure 3. Adiabatic potential ramp from 0.5 to 3.0 V to compress PFTBA ion population
HIGH MAGNETIC FIELD ELECTROSPRAY SOURCE FOR FTMS

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Among the variety of techniques available for sample ionization, electrospray ionization (ESI) is unique in that it is an extremely soft technique which produces little or no ion fragmentation and it produces multiple charges on single ions. This lowers the mass-to-charge ratio of large ions such that they can be detected by mass analyzers with a modest mass range. With high mass multiply charged ions, several peaks can occur within a single amu window and high resolution is required to adequately resolve individual charge states. FTMS may prove to be a suitable complement to ESI for a variety of reasons including routine ultra-high mass resolution, the ability to do ion molecule reactions in the trapped ion cell, and important for protein sequencing, the ability to do MS/MS. To be presented here is a novel ESI source for FTMS in which ion formation and desolvation occur in the high magnetic field of a superconducting magnet. This is made possible by a pumping configuration which utilizes multiple concentric vacuum chambers. Also to be presented is the use of a skimmer capillary as an alternative to conventional skimmer assemblies. The advantage of this approach is improved differential pumping which can be particularly important with the ultra-low pressure conditions required for FTMS detection. Preliminary results to be presented compare kinetic energy spreads for ions exiting skimmer and skimmer-capillary interfaces. This data is will be useful in efforts to efficiently trap ions for detection in the FTMS trapped ion cell.

Presented in Figure 1 is the instrumentation being developed for ESI/FTMS. As will be discussed, the ionization occurs within a 3/4" probe which is inserted into the strong magnetic field within 13 cm of the analyzer cell. Multiple stages of pumping permit 10^{-8} torr pressures to be obtained in the analyzer trapped ion cell. The vacuum chamber is positioned in a 3.0T superconducting magnet. The FTMS experiment will be conducted with components from the Extrel FTMS-2000 including dual cell, analog electronics, cell controller, and data station.

One unique feature of this interface is the formation of electrospray ions within the strong magnetic field in order to simplify the necessary ion optics. This requires that some means be found to reduce pressures from atmosphere to 10^{-8} torr within a few cm length of the magnet solenoid. As shown in Figure 2, this is accomplished by using a probe to insert the ESI assembly into the center of the magnet. Three stages of differential pumping through multiple stages of concentric vacuum tubes of increasing diameter reach mtorr, 10^{-4} torr, and 10^{-6} torr pressures, and finally the Extrel dual cell conductance limit is used to achieve a final two orders of magnitude reduction in pressure to 10^{-6} torr. The use of multiple concentric vacuum tubes is an ideal approach to pressure reduction in confined spaces such as the FTMS magnet solenoid. Their use for other high pressure source interfaces to FTMS To date, the vast majority of high pressure ionization sources which have been coupled to FTMS have been done through the fringing field. Ions are consequently injected into the trapped ion cell with electrostatic focusing optics or quadrupoles operating in the rf only mode. These injection techniques are often prone to significant ion loss due to the magnetic mirror effect. In order to overcome the adverse effects of fringing field injection we have designed a high magnetic field ESI source. The ESI probe in Figure 3 is modeled after the ESI source designed by Chait, Katta, and Chowdhury. The heated capillary serves to desolvate the electrospray aerosol without the additional gas load associated with countercurrent gas flow. The ESI probe is translatable such that the end of the desolvating capillary can be positioned within 13 cm of the analyzer cell.

Once an ion has been formed, in order for it to be trapped and retained in the FTMS cell it must have a kinetic energy along the z-axis which is less than the depth of the potential well in the trapped ion cell. In order to evaluate the kinetic energy of ESI generated ions, retarding grid studies were undertaken to assure that the kinetic energy of the ions was appropriate for FTMS detection. Figure 4 shows kinetic energy profiles utilizing two different source configurations. Figure 4a demonstrates that when a skimmer cone is placed beyond the desolvating capillary the kinetic energy of the ions is directly controlled by the potential applied to the skimmer, thus...
trapping potentials and kinetic energies can be selected for maximum trapping efficiency. If a 6 cm x 700 μm I.D. stainless steel capillary is used in place of the skimmer cone, the pressure beyond the skimmer capillary drops an order of magnitude but a much broader distribution of ion energies results.

Figure 1

Figure 2

Figure 3

Figure 4
ISOLATED DUAL TRAPPED ION CELL ASSEMBLY FOR FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

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The single cell FTMS experiment offers simplified and inexpensive operational advantages. However, reduced performance for many applications has prompted the evolution to more complex FTICR instruments, with the simplest of these designs consisting of adjacent cubic trapped ion cells which share a common trap plate. This dual cell offers several advantages but still does not correct an inefficient electron ionization duty cycle. This reduced ionization efficiency is important to FTICR because in dynamic sampling environments, its multiplex advantage for S/N is diminished if ionization occurs only during a small fraction of the total experiment time with the majority of the transient sample instead being discarded during execution of other events in the pulse sequence.

In our alternative FTICR dual cell design, two electrically isolated trapped ion cells are positioned in the strong magnetic field to facilitate concurrent ion formation, trapping, and detection. This new configuration exploits the magnetic field to deliver full FTICR detection capabilities in the source region and much higher transfer efficiencies. The primary advantage is a unit ionization duty cycle which maximizes the number of externally generated ions which are detected. Demonstrated S/N improvements for high resolution are greater than 30:1.

Isolated dual cell experiments were performed with the system shown in Figure 1. The vacuum chamber is housed in the bore of a 3.0 T superconducting magnet and is pumped with two 700 l/s diffusion pumps to low 10^-9 torr base pressures. The isolated source cell features an elongated rectangular trap with 5x5x10 cm dimensions which is probe mounted to permit z-axis translation from within 5 cm of the analyzer cell to the fringing field, 30 cm from the analyzer cell. Separating the source and analyzer regions is a 4 mm conductance limit mounted on a 4" diameter cylinder which, when differentially pumped, allows a pressure differential in excess of 10^2. A repeller electrode is positioned between the isolated cell and the grounded conductance limit to divert the electron beam from the analyzer cell during continuous beam experiments.

In conventional mass spectrometers, ionization and detection regions are separated to permit concurrent ionization and detection. Similarly, any FTICR spectrometer which isolates source and analyzer volumes should also accommodate continuous ionization and provide a near unit detection duty cycle. Wanczek placed such a source in the fringing magnetic field. In contrast, we placed the isolated source in the high magnetic field to substantially improve confinement time in the source cell, minimize the negative effects of the radial magnetic field, and minimize the spatial dispersion of the source ions.

One aspect of equilibration transfer which is of increasing importance as path length increases is that equilibration between the two cells does not occur instantaneously. This effect is more important for the external cell and is an additional reason the two cells should be closely spaced.

A gated transfer pulse sequence is often used with the isolated cell. Advantages include dual cell transfer efficiencies in excess of 95% and a crude mass filtering capability. The sole advantage of increasing the distance between cells is this improved time-of-flight-based mass selection. This can be exploited to select against low mass matrix or carrier gas ions associated with desorption or chromatographic detection. The cell can be altered to select different mass ions by changing the distance between the cells. Mass selection can be further improved by reducing the source cell length to provide better spatial focussing of the initial ion population.

A continuous beam pulse sequence was used to obtain EI spectra in the Nicolet dual cell and high field isolated cell. Peak shape distortion and line broadening are evident for the dual cell. In
contrast, for the isolated cell the spectra are unperturbed because the repeller grid ensures that ions are created and trapped exclusively in the isolated cell. The 100% ionization duty cycle which results translates into a signal enhancement which is proportional to the ratio of ionization duty cycles. Figure 2 shows analyzer cell spectra of PFTBA ions gated from the isolated high field cell following 5 ms and continuous beam events. The S/N improvement of 5.5 is in good agreement with the predicted improvement of 6.3 based on a ratio of duty cycles. Included in Figure 3 is the sensitivity profile for the continuous beam experiment acquired under high mass resolution conditions. The 30-fold change in slope is indicative of the sensitivity increases that are possible.

The duration of the transfer event, which distinguishes between mass selection and equilibration studies, is important to transfer efficiency. Figure 3 shows that equal numbers of ions are formed in gated and equilibrium transfers, yet relative sensitivity doubles for the gated transfer event because of a spatially focussed transfer and minimal losses due to collisions. A second factor in transfer efficiency is distance between source and analyzer regions. The five-fold increase in transfer efficiency for the high field source compared to the fringing field source is due in part to minimizing ion reflection in the radial magnetic field. Another problem with increasing the path length is greater dispersion of ions formed at different positions in the source cell. Signal intensity drops off rapidly with increasing source distance from the cell.

Figure 1. Translatable source cell

Figure 2. PFTBA

Figure 3. Comparison of S/N for Various Dual Cells and Pulse Sequences
Collision induced dissociation is the most popular of ion dissociation techniques in Fourier transform mass spectrometry. Still, any quantitative evaluation of the data is suspect because the dissociation event causes the spatial and kinetic energy distributions of ions to be significantly altered prior to detection. For example, it is not readily apparent how dissociation products are efficiently detected or transferred in a dual cell, if substantial radial displacement of the precursor ion has occurred.

In this work an observed pressure dependent mass discrimination effect in CID product spectra will be evaluated. The source of this mass discrimination, whether part of the CID process, excitation and detection, or collisional relaxation, will be discerned with variable excitation profiles.

CID was performed on parent ions formed by electron ionization in a 5 cm cubic cell in a 3.0 T magnetic field. Various combinations of swept, single frequency, and simultaneous single frequency excites were applied to manipulate radial ion motion. The argon target gas was maintained at various constant pressure below 10⁻⁵ torr. A 100 to 500 μs tickle pulse ensured essentially all parent ions achieved the maximum collision energy.

CID was performed on the parent ion of phenylether (m/z 170) to examine daughter ions at m/z 142 and 77. Figure 1 shows these pathways and their associated internal energies. In all experiments, the pressure of phenylether was maintained at 3 x 10⁻⁸ torr above background so that reactions with product ions, even at delays of 200 ms, were negligible. Shown in Figure 1 are breakdown curves for phenylether at increasing Ar pressure, with a 10 ms delay between tickle and detection. Under these conditions, on the order of ten or fewer collisions with the target gas are calculated. A pronounced trend to favor the lower energy (or higher mass) product is observed. One argument for this is that at higher pressures, ions do not achieve maximum translational energy before collision, thus favoring the lower energy dissociation pathways. This is unlikely, however, because the tickle pulse was chosen to be much shorter than the average time between collisions. We are examining instead mass discrimination effects in the FTICR detection process. These might result from mass dependant differences in relaxation, diffusion, or other manifestations of the collision process.

One tool developed to examine the source of the mass discrimination is the variable excitation profile which should yield information about the radial distribution of ions at different points in the FTMS experiment. Presented in Figure 2 are product ion excitation profiles for the m/z 77 and m/z 142 ions at different pressures following formation by CID at their breakdown curve maxima. What is observed is a dramatic shift in excitation energy maxima and reduction in signal intensity as pressure increases. Evidently, at higher pressures there is a significant loss of ions if excited to high energies (or increased cell radius) for detection. This effect is mass dependent with the lower mass ion, m/z 77, is lost in greater proportion at higher energies than the m/z 142 ion.

In order to target the actual location of the mass discrimination effect in the FTMS CID experiment, the molecular ion of naphthalene was examined with excitation profiles as various experiment parameters were altered. For example, Figure 3 presents control profiles for the ion at different pressures; the FTMS control experiment included a 3 ms delay between ion formation and detection, a 100us single frequency excite pulse, and broadband detection. Data, presented in the form of kinetic energy and excite radius, demonstrates a low pressure maximum at about 1200 eV kinetic energy. As pressure increases, the relative abundance decreases through a loss of ions at higher energy. This is the same effect observed in the CID product excite profiles in Figure 2, and therefore suggests that the CID portion of the experiment is not the source of the mass discrimination.
A delay was inserted between excitation and detection to produce the profiles in Figure 4. These results exhibit similarity to the control profiles in Figure 3. Evidently, the collision-related discrimination effect that occurs at high pressures is related to the portion of the FTMS experiment that follows excitation to higher kinetic energies (or larger radii). Suggestions for the mass discrimination in the CID profiles of Figure 2 are now more clearly defined. One possibility is that at increased radius, radial electric fields are more severe and ions diffuse more rapidly from the cell at higher pressures. However, this effect should not exhibit a mass dependence. Another possibility is that increased scattering of ions occurs at higher energies and higher pressures. If a sufficient increase in z-axis kinetic energy results, ions will be ejected axially. Because low mass ions are more severely scattered, the effect would be mass dependent. Excitation profiles at higher trap potentials do show a reduction in this form of ion loss. The shift in the profile to higher energy at higher trap potentials indicates some fraction of the more energetic ions are retained.

Figure 1

Figure 2

Figure 3

Figure 4
Infrared laser desorption/ionization (LDI)/mass spectrometry of organic molecules is characterized by formation of an abundant \((M+N)^+\) species from gas phase attachment of a proton or metal cation, \(N^+\), to the intact molecule, \(M\). This work is directed toward a better understanding of the mechanisms of ion formation and trapping for detection in the infrared LDI as applied to Fourier transform mass spectrometry (FTMS). The major finding to be presented is that the precursor ion in the cation attachment reaction is not the bare cation, but rather an adduct of the salt. For example, when KBr is mixed with organic samples, \(K_Br^+\) rather than \(K^+\) is found to react with \(M\) to produce \((M+K)^+\). The origin, properties, and reactivities of potassium halide adducts are evaluated. Assuming that adduct formation is a prerequisite for production of abundant \((M+K)^+\), LDI and FTMS conditions are then determined which maximize formation and trapping of these reactive adducts from the pure salts.

All experiments were performed with a FTMS assembled with components that constitute the Nicolet FTMS-2000. These include a 3T superconducting magnet, differentially diffusion-pumped ultrahigh vacuum chamber operating at base pressures in the mid 10^{-9} torr range and adjacent 5 cm cubic dual cells. A Spectra Physics DCR-11 Nd:YAG laser capable of 300 mJ pulses at 1064 nm was interfaced through a probe mounted fiber optic interface to the FTMS. The maximum power density achieved with this fiber optic interface was 3 \times 10^8 W/cm^2 at the sample surface. This value is based on 28 mJ, 9 ns laser pulse focused through the fiber to a spot size of about 900 \mu m on the sample surface. A demountable stainless steel probe tip with cylindrical geometry (20 mm length x 5 mm diameter) was used for all experiments. Two types of samples were prepared. In initial studies with the pure salt, KF, KCl, KBr, and KI salts were dissolved in water and sprayed onto the probe tip. For organic LDI experiments, a sample consisting of a 1:1 mixture of dilaurylthiodipropionate (DLTP) and a potassium dissolved in methanol was aspirated onto the spinning probe tip.

KBr adduct ions have been observed in LDI/TOF studies, but have not been proposed as possible precursors to the \((M+K)^+\) product. However, evidence that \(K_Br^+\) is involved in the FTMS experiment comes from a variable trapping profile in Figure 1 which shows the \(K_Br^+\) is less energetic than \(K^+\), and instead approximates the energy distribution for \((M+K)^+\). Additional work shown in Figure 2 and 3 with the pure potassium salts was conducted to better characterize kinetic energies of the ions formed by LDI. Presented in Figure 2 are the trapping potential profiles for \(K^+\) alone, formed from pure KF, KCl, KBr, or KI. In each case, beyond a threshold which differs for each salt, \(K^+\) is observed in increasing amounts as trapping potential increases. With the exception of KF, this threshold is above that used for most LDI/FTMS work. In Figure 3 both adduct and \(K^+\) are shown for KI at two laser energies. FTMS detection of adducts is observed to increase with laser power densities and explains why \((M+K)^+\) intensity in LDI/FTMS increases with laser power density. The individual salts can be distinguished by the overlap of kinetic energy distributions between \(K_Br^+\) and \(K^+\)--for example, \(K^+\) and \(K_Br^+\) profiles are almost completely resolved while \(K_Br^+\) overlaps with \(K^+\). This suggests that KI would be superior for LDI/FTMS work.

In comparing the four potassium halides, it was desirable to determine which of them generated \(K_Br^+\) in highest yield. As the mixture spectra of adducts in Figure 4 show, relative ranking in terms of peak areas for combined isotopes of each adduct is \(K_Br^+ > K_BrCl^+ > K_Br^2+\), but there is little difference in relative abundance. In considering the origin of the adduct, it is possible that intact \(K_Br^+\) from the desorption site subsequently penetrates the trapping fields and is retained for detection; alternatively, the adduct could be generated in the cell by gas phase attachment of \(K^+\) to the neutral salt or by decomposition from larger adducts. Double resonance experiments were performed to distinguish this. In the absence of an ejection event, both \(K^+\) and \(K_Br^+\) are immediately detected and at longer delays remain in a fixed proportion which is indicative that neither undergoes gas phase reactions. But, when the \(K^+\) is ejected continuously between desorption and detection, the \(K_Br^+\) remains in large abundance. This indicates \(K_Br^+\) is not formed in the cell from \(K^+\). Sample preparation is an important consideration in preferential formation of adduct in the LDI/FTMS experiment. KBr is seen to dominate at 25°C. In contrast, results are for a vacuum chamber heated...
to 170°C and maintained for several hours. In this case, K⁺ dominates under otherwise identical conditions.

Evidence that the adduct, KₓX⁺, rather than K⁺ reacts with M comes from double resonance experiments as shown in Figure 5. In Figure 5a, when K⁺ is continuously ejected to reduce space charge, abundant (M+K)⁺ is seen. In contrast, when Kₓ⁺ is ejected in Figure 5b, no (M+K)⁺ is detected which indicates it is a precursor to the cation attachment process. In order to evaluate whether the higher adducts are also involved in reactions with M, a series of double and triple resonance experiments involving K⁺, KₓF⁺, and KₓF₂⁺ reactions with DLTDP were performed. Here KₓF₂⁺ is observed to be highly reactive.

Figure 1  LDI/FTICR of DLTDP and KBr

Figure 2  Variable trapping studies of K⁺ from pure salts

Figure 3  Variable trapping profile for KI

Figure 4  LDI FTMS spectrum of a mixture of four salts at 2.0 V

Figure 5  The spectrum of Gramicidin-S and KI

a) 7x10⁷ W/cm²

b) 2x10⁸ W/cm²
Ion/molecule Reactions of Externally Produced Metal Ions
In a Quadrupole Fourier Transform Mass Spectrometer

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Department of Chemistry, University of California, Davis, CA 95616

The External source quadrupole Fourier Transform Mass Spectrometry (QFTMS) instrument, although well suited for ion/molecule reactions, has not been used for this purpose. We are currently investigating the use of the instrument built at U.C. Davis for the formation and reaction of ligated transition metal ions (Figure 1). The instrument has been developed to be versatile for use in both ion/molecule chemistry and analysis. It has both EI/CI and FAB sources. An electrospray source is currently being developed. A split foreline allows introduction of reagent gasses into the source and the analyzer region via either pulsed valves or precision leak valves. High mass range is obtained despite the relatively low (3 tesla) superconducting magnet. Cesium iodide clusters as large as m/z 16241 (CsI)62Cs+ are observed. Resolution of 41,000 for m/z 1692 (CsI)6Cs+ is obtained.1

There were two major concerns on doing metal ion chemistry in the external source instrument with ions produced from a SIMS source. The first was whether low mass ions could be effectively transported from the source to the analyzer cell by the RF only quadrupole broad band mass filter and second was whether the large presence of Cs+ from the cesium gun would simply swamp out any metal ions injected into the analyzer cell. As seen in figure 2 metal ions can be produced and transported into the analyzer cell in large abundance and in the absence of Cs+. We now routinely produce bare transition metal ions using a secondary ion mass spectrometry (SIMS) source from either the neat metal salt or a liquid (glycerol) matrix. Forming ions with this method offers an alternative to laser ablation and electron impact ionization which are currently the most commonly used technique.

The metal ions are trapped and isolated in the analyzer cell and the reagent gas pulsed into the chamber. Argon is used to translationally cool the ions before reaction. Known metal reactions such as the reaction of Fe+, Co+, and Ni+ with alkane (n-pentane) are examined and the branching ratios compared to literature results (Table 1).2 We find little difference between our results and those experiments performed in an FTMS with laser ablated ions. Cooling the ions with argon before the reaction does not significantly vary the branching ratio. There is however evidence that the metal ions are translationally excited when trapped in the analyzer cell. A comparison in the reaction of Fe+ to CH3S-SCH3 with (Figure 3b) and without Ar (Figure 3a) precooling shows dramatic differences in the products formed. By collisionally cooling the metal ions with Ar before the reaction only one ionic species is observed corresponding to Fe(CH2-S)+. Without argon at least four major products are obtained including highly endothermic reactions such as charge transfer (to form the methyl/disulfide cation) and formation of FeSCH3+. Our interest in this reaction is the use of metal ions as a Cl gas to locate the position of S-S bonds in bio-organic molecules.

The SIMS source is ideal for the formation of simple and complex ligated metal ions. These ions will be used to react with organic compounds to determine the effect of ligation on the reactivity of the metal ions. We plan to produce metal complexes with varying denticity as well as types of coordinating atoms. So far ligated metals from simple Co(H2NCH2CH2NH2)+ to metal octaethyl- and tetraphenylporphyrins (M= Fe, Co, Ni, Zn) are readily produced.

Figure 1. See text.

Figure 2. 

Figure 3. Reaction of $\text{Fe}^+$ with $\text{CH}_3\text{S}-\text{SCH}_3$.

Table 1. Branching ratios

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<th>Fe$^+$</th>
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Reference

Electrospray ionization (ESI) has made remarkable strides recently as a very soft ionization method for proteins. However, the application of ESI to complex mixtures and the unambiguous determination of mass and charge of daughter ions in MS/MS requires high mass resolution. Fourier transform mass spectrometry (FTMS) can provide that resolution given that detection occurs at pressures below \(1 \times 10^{-1}\) torr. This requires two orders of magnitude further pressure reduction beyond what is typically required in a quadrupole instrument, and requires that the ions be injected from an external atmospheric pressure source through the fringe field of a 3 tesla superconducting magnet. Previous workers (K. Henry and F. W. McLafferty, and J. Shabanowitiz and D. F. Hunt, 38th ASMS, June, 1990, Tucson, AZ) have used a quadrupole operated in the RF-only mode to guide ions to the cell, and have detected multiply charged ions from peptides as large as bovine serum albumin, MW 66,000.

Our design is shown in the figure, and consists of two stages of differential pumping before the analyzer cell of a Nicolet (now Extrel) FTMS-2000. Ions are electrostatically focused through a conductance limit and then into the magnet at 500 eV, followed by deceleration just prior to the cell. The ion optics were designed with the aid of MacSimion (D. McGilvery and R. Morrison, Monash University, Centre for High Resolution Spectroscopy and Opto-Electronic Technology). At present, while electrospraying water-methanol-acetic acid, 50 picoamps reach the skimmer, up to 5 picoamps pass through, and of this, up to 3 picoamps reach the FTMS cell. Pressures are 0.4 torr before and 4x10^{-4} torr after the skimmer, respectively, and 1x10^{-5} torr in the analyzer cell region. This is with a 4 mm orifice into the cell region. Reducing this and the skimmer orifice while increasing the diameter of the capillary should lower the pressure by a decade and increase the current by at least a decade. This increased current, coupled with improvements in the kinetic energy distribution of the transmitted ions, should allow ions to be trapped and mass analyzed in the FTMS.
EXTERNAL SOURCE AND ION TRANSPORT ASSEMBLY

SOURCE CELL

DECELERATOR TUBE

ANALYZER CELL

FLIGHT TUBE

GATE VALVE

FLIGHT TUBE

GATE VALVE LENS

SKIMMER AND LENS ASSEMBLY

CAPILLARY

GOLD COATING

ELECTROSPRAY NEEDLE

TO 700 L/S DIFFUSION PUMP

TO 700 L/S DIFFUSION PUMP

TO 1200 L/S DIFFUSION PUMP

TO 765 L/M MECHANICAL PUMP

THERMOCOUPLE GAUGE TUBE
NOVEL REARRANGEMENT OF SiH(CH₃)(C₂H₅)⁺ TO Si(CH₃)₃⁺  

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Organosilicenium ions (SiR₃⁺) are highly reactive in the gas phase with a number of interesting rearrangements observed[1]. The facile elimination of ethene for CAD of Si(CH₃)₃⁺, process 1, is particularly intriguing[2]. We have studied the mechanism of rearrangement of simple organosilicenium ions by using Fourier transform mass spectrometry (FTMS) in conjunction with isotopic exchange/labeling and CAD. Our results suggest that SiH(CH₃)(C₂H₅)⁺ can readily rearrange to the more stable Si(CH₃)₃⁺ ion, presumably by a concerted 1,2-methyl/1,2-hydrogen migration (dyotropic rearrangement), process 2[3].

Si(CH₃)₃⁺ + C₂H₄ → SiH⁺ + CH₃CH₂⁺  

(1)

Si(CH₃)₃⁺ is inert with ethene-D₄ however, SiH(CH₃)₂⁺ undergoes exclusively one facile H/D exchange, process 3. Both Si(CH₃)₃⁺ and SiH(CH₃)₂⁺ are inert with ethene-¹³C₂. The H/D exchange in process 3 presumably proceeds by reversible ethene insertion/β-hydrogen migration, process 4. The above results suggest that SiH₂(CH₃)⁺ should yield two exchangeable hydrogens with ethene-D₄. Surprisingly, all five H's in SiH₂(CH₃)⁺ are rapidly exchanged with C₂D₄. The H/D exchange for SiH₂(CH₃)⁺ must proceed via two distinct mechanisms, one where the Si-H's are exchanged (process 4) and one where the methyl-H's are exchanged. In addition, SiH₂(CH₃)⁺ undergoes facile carbon atom exchange with ethene-¹³C₂.

How can these dramatic differences between these seemingly similar silicenium ions be rationalized. We propose that this scrambling for SiH₂(CH₃)⁺ with labeled ethene involves initial insertion into an Si-H bond yielding an ethylmethylsilicenium ion. This activated ion subsequently undergoes concerted 1,2-methyl/1,2-hydrogen migration (dyotropic rearrangement), presumably via a four centered transition state, 1, that is a thermally
allowed \([O^+ + \text{Si-CH}_2]\) process. 1 requires that the methyl group migrates with inversion. \(\text{Si-CH}_3\) is not sterically hindered and does not require the involvement of d orbitals or the empty p orbital on silicon. The facility of the isotopic exchange demands a low barrier for the rearrangement, process 2. The driving force for process 2 is the increase thermal stability of \(\text{Si(}\text{CH}_3\}_3\)\(^+\) (~17 kcal/mol).

We now have to address the fact that reaction of \(\text{SiH(CH}_3)_2\)\(^+\) with labeled ethene does not result in isotopic exchange of the methyl groups. Here, initial ethene insertion would generate an ethyldimethylsiliconium ion. A thermally allowed \([O^+ + \text{Si-CH}_2]\) can proceed in analogy to \(\text{H}_2\) where the migrating hydrogen atom is replaced with a methyl group. Again, this concerted migration would not be sterically constrained. There must, however, be a prohibitive barrier for this rearrangement since it is not observed. We believe that there is a prohibitive barrier to this process, presumably do to the smaller size of the sp\(^3\) hybrid orbital on the methyl relative to the H 1s orbital.

\(\text{SiH(Cl)(CH}_3\}_3\)\(^+\) behaves analogously to the \(\text{SiH(CH}_3)_2\)\(^+\) ion yielding only one H/D exchange with ethene-D\(_4\).

TRIMETHYLSILYL GROUP MIGRATIONS IN ELECTRON IMPACT
MASS SPECTRA OF CHOLESTEROL OXIDES:
MECHANISTIC INTERPRETATIONS AND PRODUCT CHARACTERIZATION

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It is well known that the migratory aptitude of the trimethylsilyl (TMS) group is high and that it will migrate to a suitable electron rich site via a wide range of intermediate ring sizes. In fact, TMS migrations have been shown to be more competitive than hydrogen migrations. As expected, such migrations have been observed in the EI mass spectra of semi-rigid sterols where the TMS group and the electron rich site are arranged such that a 6-membered ring transition state is clearly available. Brooks and co-workers have, however, demonstrated the migration of the TMS group in the EI mass spectra of a number of \(\Delta^3\)-3-fraction-stereoids. The migration was proposed to occur over 5 atomic centers in a conformationally rigid system and precede A-ring cleavage. We report a variation of this decomposition in the EI mass spectra of cholesterol autoxidation products. The TMS group has been observed to migrate to electron rich functional groups in the 6 and 7 position of the B-ring. Tandem mass spectrometry was used to examine the nature of the ion produced from the intramolecular TMS group transfer. High and low energy collision induced dissociation (CID) and isotope labelling were employed to confirm and elucidate this process.

All high energy CID experiments were performed on a VG-70/250SE double focussing mass spectrometer using B/E linked scanning. The laboratory collision energy was 8KV and the collision gas was Helium. All low energy CID experiments were performed on a VG-QUATTRO triple quadrupole mass spectrometer. The laboratory collision energy was 70V and the collision gas was Argon.

TMS migration has been observed in the EI mass spectra of cholestan-5\(\beta\),6\(\alpha\)-epoxy-3\(\alpha\)-ol (\(\alpha\)-epoxide), cholestan-5\(\alpha\),6\(\alpha\)-epoxy-3\(\alpha\)-ol (\(\alpha\)-epoxide), 5\(\alpha\)-cholestan-3\(\alpha\)-ol-6-one (6-keto), 5\(\alpha\)-cholestan-3\(\alpha\)-ol-7-one (7-keto), and cholestan-3\(\alpha\),5\(\alpha\),6\(\alpha\)-triol (triol). The TMS migration is believed to precede A-ring fragmentation and result in the loss of 71 amu in the case of the triol, 6-keto, and epoxides. The same is believed to occur in the case of the 7-keto. The TMS migration, however, is instead associated with the loss of 69 amu. The mechanism for the 6-keto, epoxides, and triol are thought to be the same, while that for 7-keto is thought to be different. Two different structures for the resultant m/z 403 ion are, therefore, expected. They are shown in Figures 1a and 1b.

The migration of the TMS group as well as the proposed mechanisms are in agreement with mass shifts in the EI spectra of the 2,2,4-\(\Delta\)-, the d\(9\)-TMS, and the \(18\)O labelled analogues. The high energy CID spectra of the products of migration in the epoxide, 6-keto, and the triol also support the proposed mechanism. The CID spectrum of the product ion from the triol is shown in Figure 2 and is representative of the others. Based on the proposed mechanism the 6-keto, triol, and \(\alpha\)-epoxide are expected to yield the same fragment ion structure. The similarity of the CID spectra from these compounds supports this expectation. The fragmentation is primarily confined to loss of the side chain and cleavage through the rings C and D. Furthermore, the limited amount of cleavage involving the TMS group or the B-ring indicates charge stabilization in that region. In contrast, the product of TMS migration in the 7-keto is expected to have a structure in which the charge delocalization is not so extensive. That a structural difference exists is apparent in the comparison of the CID spectrum from 7-keto in Figure 3 with Figure 2. The striking suppression of all but one of the charge remote cleavages and the intensity of the ion at m/z 313 resulting from loss of trimethylsilanol is indicative of a structure such as that proposed in Figure 1b. The loss of TMSOH is expected to be intense since it would result in aromatization of the B-ring.

The difference between the low energy CID spectrum of 7-keto and those from the 6-keto, epoxide, and triol is still pronounced, with the 7-keto yielding an intense peak at m/z 313 from the loss of TMSOH. In general, however, the low energy fragmentation appears to yield primarily low mass ions below mass 200. This suggests that at low collision energy rearrangement as well as charge induced fragmentation is favored.

In conclusion, the migration of the TMS group to various electron rich sites on the B-ring has been confirmed by isotope labelling and B/E linked scanning. Furthermore, high and low energy CID of the product has provided useful information regarding the mechanism of the migration / fragmentation sequence.
FIGURE 1A
Product Ion for Epoxydes, 6-Keto, and Triol

\[
\text{TMS} \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{CH}_2 \\
\text{m/z 403}
\]

FIGURE 1B
Product Ion for 7-Keto

\[
\text{TMS} \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{CH}_2 \\
\text{m/z 403}
\]

FIGURE 2
High Energy CID of m/z 403 from Cholestan-triol

FIGURE 3
High Energy CID of m/z 403 from 7-Ketocholestenol

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A CID AND METASTABLE ION STUDY OF M/Z 84 IONS FORMED FROM 2-ETHYLPIPERIDINE, N-(2-
HYDROXYETHYL)PYRROLIDINE, N-METHYL-2-HYDROXYMETHYLPIRROLIDINE AND n-
BUTYL(BENZYLIDENE)AMINE

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Relatively few mass spectrometric studies have been made for organic Schiff bases as compared with other classes of organic compounds. Many interesting rearrangement decomposition ions exist in the El mass spectra of alkyl(benzylidene)amines, C6H5CH=N-R, for example, the m/z 84 ion (loss of C6H5) formed from n-butyl species (R = n-C4H9, compound I) and the m/z 160 ion (loss of CH3, when R is n-pentyl; loss of C2H5, when R is n-hexyl, etc.). Two independent mechanisms have been postulated for the formation of the m/z 84 and m/z 160 ions (1)

An alternative mechanism involving competing decompositions might be operative:

This study compared the daughter ion spectra (metastable and collisionally induced decompositions (CID)) of m/z 84 ions formed from compound I and those formed from electron ionization of (a) 2-ethylpiperidine, (b) N-(2-
hydroxyethyl)pyrrolidine and (c) N-methyl-2-hydroxymethylpyrrolidine to provide the insights of the ions structure.

Experiments were performed with a VG 70-VSEQ triple sector (E/B/q/Q) instrument. Samples were separated from a mixture with an HP 5890 gas chromatograph. Mass spectrometer conditions: source temp. = 205 °C; high energy CID in the first field free region with He as the collision gas at 60 % attenuation; low energy CID in the third field free region, 15 eV collision energy, -6 x 10^-4 mbar Xe. Samples: (a)-(c) commercially available, compound I was prepared from reaction of equal molar amounts of benzaldehyde and n-butylamine.

The formation of m/z 84 in the El mass spectrum of compound I involves a rearrangement process (1). Several structures are possible for this m/z 84 ion, i.e.

(a1)-(c1) give easily distinguishable daughter ion spectra from linked scan (metastable decompositions), high energy CID and low energy CID. Ionization energy does not have any significant effect on the spectra. The daughter ion spectra are not available for ion (d1). The m/z 84 ion formed from compound I gives daughter ion spectra similar to (a1). Low energy CID shows differences in the relative abundances of m/z 54, m/z 55 and m/z 69 etc. At 70 eV ionization energy, the differences are fairly small, ca. 10 % of m/z 56. The differences increased when ionization energy was decreased. This result suggests that at high ionization energy, the m/z 84 ion formed from compound I may have main structure of (a1), instead of our suggested structure (b1). At low ionization energy, another structural isomer may also formed. One possible structure is (d1), the previously suggested structure, since any linear combination of (a1) with (b1) or (c1) ion will not fit the CID data.

It was found that low energy CID spectrum of m/z 84 ion formed from n-butyl(acetylidene)amine, C6H5CH=N-C4H9, is indistinguishable from that of (a1). The formation of stable (a1) ions is a common process for both C6H5CH=N-C4H9 and C6H5CH=N-C4H9. Further studies are necessary to determine the structure of m/z 160 ions in higher alkyl species.
Suggested mechanism for the formation of (a1) from compound I:

Reference:

Low energy CID spectra of m/z 84 ions.
Reactions of V⁺ With Small Heteroatom Containing Molecules
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Introduction
We report here on the reactivity of atomic vanadium cation with water, small alcohols, and amines. V⁺ has been previously observed to activate C-H bonds in hydrocarbons. We have studied the product distributions, kinetic isotope effects, and subsequent reactions for these small molecules and observed some expected and some interesting behavior.

Experimental
All experiments were performed on a Fourier transform ion cyclotron resonance mass spectrometer. Atomic vanadium cations were generated by focusing the fundamental output of a Spectra-Physics pulsed Nd:YAG laser (pulse energies of ~135 mJ) on pure metal blocks. Reagent pressure in the vacuum chamber was ~5-8x10⁻⁶ torr (uncorrected). After initial ionization, the metal ions were trapped for 200-400 msec to allow for collisional and radiative cooling. After the cooling period the ions were isolated by swept double resonance ejection pulses. This cooling period should allow for nearly complete translational cooling (35-70 collisions).

Results
The reactions of V⁺ react with water via three primary reaction pathways, formation of VO⁺, VOH⁺, and VH₂O⁺, with formation of the oxide being the dominant pathway. The reaction to produce VH₂O⁺ exhibits an isotope effect, indicating the complex formed in the low pressure environment of the FT-ICR has a bond inserted structure. The overall reaction exhibits a normal kinetic isotope effect. The secondary reactions differ from the primary reactions in that VOH⁺, which is the most reactive primary ion, produces predominantly V(OH)₂⁺ with only a small amount of V(OH)O⁺. This is due to electronic saturation effects, as shown in scheme I. V(OH)₂⁺ subsequently attaches three molecules of water, reaction (1). The primary reaction to form VOH⁺ implies D(V⁺-OH)>118 kcal/mol.

The reactions with methanol produce the metal oxide, metal hydroxide, and metal alkoxide. A partial breakdown of the reactions with V⁺ is shown in Figure I. In contrast to methanol which does not undergo a 1,2 dehydrogenation pathway in the primary reactions, ethanol reacts via a 1,2-dehydrogenation mechanism to produce V(acetaldehyde)⁺. Methylamine reacts to (1) generate the amide (2) via a 1,2 hydrogen migration to produce the imine (VNH⁺). Similar behavior is observed for ethylamine. As for all molecules reported in this study, extensive subsequent reactions are observed with V⁺.
\[ \text{Scheme I} \]
A mass spectrum can be considered to be the result of a kinetics experiment. It is a convolution of a series of competitive and consecutive reactions that depend upon the internal energy deposited during ionization and the time-frame during which reactions can occur. The complexity of many mass spectra generated by electron ionization results from the fact that a range of internal energies are deposited by the ionization event and that reactions can occur over six or more orders of magnitude of time, i.e. from $10^{10}$ to $10^{6}$ seconds on traditional beam (sector and quadrupole) instruments. For ion trapping instruments this time range is increased by several more orders of magnitude since the mass analysis generally occurs some milliseconds or more after the ionization event in these instruments. In general, nevertheless, EI spectra on trapping instruments and beam instruments are quite similar if the experiments are done appropriately.

The same kinetic considerations apply to MS/MS experiments. However, the differences in time-scale between trapping and beam instruments may have more of an effect upon MS/MS spectra. There are two potential effects resulting from the different time scales. The first of these is the internal energy/structural distribution of the parent ions. In the trapping instruments parent ions must be stable for milliseconds as opposed to microseconds for beam instruments. Therefore parent ions that are "metastable" in beam instruments are unstable in trapping instruments. For ion trapping instruments this time range is increased by several more orders of magnitude since the mass analysis generally occurs some milliseconds or more after the ionization event in these instruments. In general, nevertheless, EI spectra on trapping instruments and beam instruments are quite similar if the experiments are done appropriately.

There are two possible effects of the difference in the dissociation "time-window". The first of these is that the much shorter time window in beam instruments may favor observation of faster reactions such as simple cleavages as opposed to rearrangement reactions. The other possible effect is that the several orders of magnitude longer dissociation time-window in trapping instruments may allow much higher MS/MS efficiencies due to the longer "integration" time for the dissociation.

Figure 3 shows an example in which the faster, simple cleavage reactions are substantially enhanced in the TSQ MS/MS spectrum. The parent ion in this example is (M-H)$^+$ from N,N-dimethylaniline. The simple cleavage ions are m/z 105 (loss of methyl radical) and m/z 77 (loss of CH$_3$N+). All the other ions are the result of rather complex rearrangements. These simple cleavage ions dominate the MS/MS spectrum if more internal energy is put into the parent ion by using Ar instead of He as the collision gas.

While it can be argued that the simple cleavage ions are enhanced in the beam instrument due to internal energy considerations, the relative intensity of the rearrangement ions is substantially different between the two instruments and these relative intensities are very insensitive to collision energy in each instrument. This is shown in Figure 4 which are plots of the relative intensity of three major rearrangement ions in the ion trap experiment and absolute intensity of these ions in the TSQ 700. Another pertinent difference in the MS/MS spectra of this species obtained on the two different instruments is the MS/MS efficiency. The MS/MS efficiency for the ion trap is an order of magnitude greater than that for the TSQ.
As parent ions get larger and larger the dissociation time-window may be even more important since there will be an ever increasing kinetic shift due to the increase in the number of degrees of freedom. A specific case in which this may be significant is in the dissociation of peptides. While the critical energy for a given type of dissociation (i.e. a,b,y, etc.) should not be dependent upon the size of the peptide, the amount of excess internal energy required for the dissociation to occur within the dissociation time-window will increase with increasing mass. Our observation with small protonated peptides is that very similar dissociations are observed in the ion trap as with quadrupole instruments but that the ion trap MS/MS efficiency usually approaches 100% whereas the quadrupole efficiencies are typically a few percent. The most striking difference we have thus far observed is for leucine enkephalin-Arg. While spectra of this compound obtained on beam instruments are consistent with the general observation that a terminal arginine can drastically affect the dissociation pathways, in the ion trap MS/MS spectrum the dissociation pathways differ little from those of leucine enkephalin, with all the typical sequence ions being observed. Additionally, the ion trap has ≈100% MS/MS efficiency while the beam instruments have efficiencies < 10%.

The interaction of \( p \)-benzoquinone (Q) with electrons has been studied extensively in the gas phase. A particularly interesting characteristic of Q is that it undergoes electron capture, reaction 1, extremely slowly, despite its high electron affinity (1.9 eV). We have investigated two gas phase photochemical reactions involving Q, methyl-\( p \)-benzoquinone (MQ), 2,6-dimethyl-\( p \)-benzoquinone (DMQ), and tetramethyl-\( p \)-benzoquinone (TMQ), and the molecular anions of these molecules at atmospheric pressure using a modified electron capture detector (ECD) and atmospheric pressure ionization mass spectrometry (APIMS). Electron photodetachment (PD), reaction 2, from the molecular anions is observed in the UV-visible spectral range. In addition, electron capture enhancement (ECE), reaction 3, by the neutral molecules is observed when light of specific wavelengths is passed through the ECD. Although these two processes occur simultaneously over a portion of the UV spectrum, we have developed a method that allows the study of either of these reactions without interference from the other. Therefore, the ECE spectra of the quinone molecules and the PD spectra of the quinone anions can be independently determined and are reported here. We also report APIMS measurements which show that within the ECD, a photochemical dimerization of Q takes place which is responsible for the ECE by these molecules.

The modified ECD is shown in Figure 1. The compounds are introduced to the ECD by means of a gas chromatograph. A 1000 W Xe arc lamp, an optical chopper operated at 5Hz and a high throughput monochromator are used to focus a chopped light beam with a 20 nm bandwidth into the ECD cavity. The current signal from the ECD is processed by the electrometer and is sent to pen 1 of the recorder and also to the lock-in amplifier (LA) as shown. In the LA, the electrometer signal is mixed with the square waveform coming from the chopper. The phase angle offset of these two waveforms may be adjusted manually or optimized internally by the LA. The resulting product is sent to pen 2 of the recorder. Thus, pen 1 provides a response, \( \delta_{\text{ECE}} \), that is closely related to a normal ECD response, while pen 2 provides a response, \( \delta_{\text{PD}} \), due to any photochemical processes occurring within the ECD.

At wavelengths near 300 nm, both PD and ECE occur within the ECD simultaneously. We were able to obtain the PD and ECE spectra independently and without interference from each other by careful manual control of the LA phase angle offset (\( \delta \)). Typical chromatograms are shown in Figure 2. Beneath the chromatograms are included simulations of the two waveforms sent to the LA and the manually chosen \( \delta \) for that measurement. In A, the ECE signal is maximized with a \( \delta \) of -90° and in B, it is zeroed with a \( \delta \) of 0°. Thus, when the PD signal is maximized, the ECE signal is zeroed, and vice versa. The reason for the difference in \( \delta \) for the two processes is that the PD rate constant of 300 s\(^{-1}\) is fast when compared with the chopping frequency of 5Hz, while the ECE signal changes very slowly. At 5Hz, the PD signal assumes the shape of a square wave and closely tracks the reference square wave. The optimal \( \delta \) for PD is therefore 0°. However, the triangular waveform of the ECE signal must be shifted by -90° to obtain optimal mixing with the reference square wave in the LA.

The PD spectra of the molecular anions are shown in Figure 3. In A, in addition to the PD spectrum for Q\(^{\prime} \), the known condensed phase absorption spectrum of Q is shown. The peaks in the PD spectrum resemble those in the absorption spectrum closely, because in the regions where Q\(^{\prime} \) absorbs strongly, resonance PD occurs. The ECE spectra of the molecules are shown in Figure 4. In Figure 5, the known condensed phase absorption spectra for the neutral molecules are shown for comparison with the ECE spectra. All of the molecules show the most pronounced ECE at \( \lambda_{\text{ECE}} \).

MQ and DMQ also show weak ECE at X, which show that within the ECD, a photochemical dimerization of Q takes place which is responsible for the ECE by these molecules. The mechanism responsible for ECE was elucidated using negative ion APIMS. The setup of the APIMS experiment is shown in Figure 6. The optical setup is identical to the previous experiment except that no optical chopper is used. The ion source is a modified ECD similar to the ECD in the previous experiment. For better signal intensities, a low resolution was used during acquisition of mass spectra. The mass spectrum of Q acquired with the arc lamp turned off is shown in Figure 7A and exhibits only one peak at m/z 108, which corresponds to the molecular ion, Q\(^{+} \). Figure 7B shows the mass spectrum taken with the arc lamp turned on and the monochromator set at 240 nm. The base peak at m/z 108 corresponds to Q. The large peak at m/z 216 is thought to be due to Q\(^{\prime} \), the negative ion of a dimer of Q. The mechanism for formation of Q\(^{\prime} \) is believed to be a simple photochemical 2+2 cycloaddition between two molecules of Q. Q\(^{\prime} \) is thought to have an electron capture rate coefficient that is several orders of magnitude greater than that for Q. The ECE response is therefore explained by reaction 4, in which Q is excited to Q\(^{\prime} \) by absorption of a UV photon. It then dimerizes with a ground state Q to form Q\(^{\prime} \), which rapidly captures an electron. It is believed that ECE by MQ, DMQ, and TMQ is also due to formation of dimers that have higher electron capture rate coefficients than the monomers.
Figure 1. Modified ECD used for photodetachment and ECE studies.

Figure 2. Chromatograms of p-benzoquinone (Q) with phase angle offset (θ) shown below. A & B show ECE response at 230 nm. C & D show PD response at 315 nm.

Figure 3. Photodetachment spectra of the quinones.

Figure 4. Electron capture enhancement (ECE) spectra of the quinones.

Figure 5. Absorption spectra in n-hexane of the quinones from Braude, J. Chem. Soc. 1945, 490-497.

Figure 6. APIMS setup.

Figure 7. In A, normal negative ion APIMS spectrum of p-benzoquinone. In B, ion source is irradiated with 240 nm light.
Our research efforts have been directed at understanding the mechanism of one-electron reduction of polychlorodibenzo-p-dioxins (PCDDs) under electron capture negative ionization mass spectrometry (ECNI-MS) conditions. We have shown, for example, that there exist a linear correlation between the branching ratio $\log \left( \frac{[M-Cl]}{[Cl]} \right)$ and the LUMO energy for the PCDDs and polychlorinated dibenzofurans (PCDFs) under ECNI-MS conditions (1). A subsequent study has also shown that a linear correlation exists between the branching ratio $\log \left( \frac{[M-Cl]}{[Cl]} \right)$ and the energy of the products formed when PCDDs were subjected to ECNI-MS conditions (2). It was hypothesized that the one-electron reductive dehalogenation of PCDDs under ECNI-MS conditions could be regioselective (3). Testing this hypothesis required the synthesis of chlorine-37 labeled PCDDs (4).

Isotope enrichments were calculated using an in-house program. The algorithm seeks to minimize the Chi-square between a synthetically generated isotopic pattern based on a modified binomial expansion and the experimental data (4,5). The relative abundance of chloride ion ejected from each position was determined from the observed $^{35}Cl/^{37}Cl$ ratio by solving the following simultaneous equations (5).

$$ax + cy = I_{35}$$
$$bx + dy = I_{37}$$

$I_{35}$: Relative intensity of $^{35}Cl$ in the NCI mass spectrum.
$I_{37}$: Relative intensity of $^{37}Cl$ in the NCI mass spectrum.
$c$: Relative abundance of $^{35}Cl$ at the labeled position.
$d$: Relative abundance of $^{37}Cl$ at the labeled position.
$a$: Relative abundance of $^{35}Cl$ at the unenriched positions.
$b$: Relative abundance of $^{37}Cl$ at the unenriched positions.

$$a = 0.757n$$
$$b = 0.243n$$

$n$: Number of natural chlorines present in the molecule.
$x$: Relative chloride ion loss from unenriched position(s).
$y$: Relative chloride ion loss from the labeled position.

Our results clearly show that the one-electron reductive dehalogenation of PCDDs under ECNI-MS conditions is regioselective. The process does not exhibit any primary kinetic isotope effect. This is illustrated by the equal chloride ion loss from the unenriched and labeled position in the symmetrical dichlorodibenzo-p-dioxin (DCDD) isomer (see Figure 1).

The most interesting results are those found for the reductive dehalogenation of 1,2,3,7,8-PCDD and 1,2,3,4,7,8-HexCDD. Under ECNI-MS conditions, chloride ion loss from a position (ortho to the oxygen) is predominant in both cases. Thus, the reductive dehalogenation of 1,2,3,7,8-PCDD yields the extremely toxic 2,3,4,8-TCDD; while the 1,2,3,4,7,8-HexCDD would afford 1,2,3,7,8-PCDD upon loss of a chlorine atom. This tends to support the view that the reductive dehalogenation of toxic PCDDs does not necessarily lead to a less toxic product.

Acknowledgement: We gratefully acknowledge the support from the National Institutes of Health (NIEHS 210 and NIEHS 40).

Fig 1. Regiospecific chloride ion loss under ECNI-MS conditions.
COMPEITION BETWEEN S_N2 AND PROTON TRANSFER; 
ANIONS REACTING WITH THE CHLOROMETHANES

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Department of Chemistry, Harvard University, Cambridge, MA 02138.

Proton transfer (PT) and nucleophilic substitution (S_N2) are among the most important chemical transformations. We were interested in exploring the competition between PT and S_N2 reactions and the effects of anion structure and reaction exothermicity. The chloromethanes were chosen because the simplicity of this neutral system; there are generally no energetically accessible channels available other than PT and S_N2. By combining the data for reactions of monochloromethane which have been extensively studied [1,2], the complete picture of the chloromethane system can be assembled. The reactions were carried out in a flowing afterglow at room temperature.

For dichloromethane (CH_2Cl_2) reacting with anions of decreasing basicity, the fraction of S_N2 product remains low (~5%) until PT is close to thermoneutral and the S_N2 process becomes predominant (Figure 1). Delocalized anions react slower than localized anions with similar basicity but the PT/S_N2 ratio are essentially the same.

In the case of chloroform (CHCl_3), independent of the nature of the anions, proton transfer and the adduct formation when PT is endothermic, are essentially the only observed processes. Very slow formation of S_N2 product is observed only when PT is shut off completely by reaction endothermicity.

In the reaction of anions with carbon tetrachloride (CCl_4) where there is no proton transfer channel available, the formation of CI^- is also very slow except for NH_2^- and C_3H_5^-.

A new and interesting channel opens up which is the substitution at Cl. This new channel is very fast and constitutes the major products for NH_2^-, C_3H_5^-, O^- and EtO^- while in the case of OH^-, F^- and HS^-, substitution at Cl is quite slow and the major products are the formation of the adduct (Table 1).

Comparing the reaction of all chloromethanes revealed that S_N2 process competes with PT favorably in CH_3Cl, less favorably in CH_2CH_2 and poorly in CHCl_3 (Table 2 shows a typical set of data). Because exothermic PT are generally fast, the decrease of S_N2 yield on increasing Cl substitution must reflect the decrease in S_N2 efficiency which is supported by reactions of CCl_4 with anions other than NH_2^- and C_3H_5^-.

This decrease of S_N2 efficiency with increasing Cl substitution may have electronic origin or more likely, steric origin caused by the bulky electron crowd around Cl.

The results of the reaction of CH_2Cl_2 with anions reflect the interplay among the following factors: (1) PT has lower kinetic barriers than S_N2; this dictates the low fraction of S_N2 product. (2) PT is generally less exothermic than S_N2 for the same anion which causes the sharp increase in the S_N2/PT ratio when PT becomes thermoneutral. (3) Electron delocalization in the anions slows down both reaction channels.

The observation of the facile process of substitution at Cl in CCl_4 is very interesting because this is an uncommon type of reaction and the leaving group here is a carbon base. The fast formation of Cl^- in the reaction of CCl_4 with NH_2^- and C_3H_5^- may be due to the dissociative electron transfer rather than a S_N2 process (Figure 2). The abundant amount of adduct formed in reaction of OH^-, RO^-, F^- and HS^- with CCl_4 may serve as a convenient way of preparing those clusters for CAD experiments.

Acknowledgement

This work was supported by an NSF-PYI award to JJG and by the ACS-PRF.

References


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Table 1. Results of \( \text{HA}^+ + \text{CCl}_4 \).

<table>
<thead>
<tr>
<th>Adduct</th>
<th>Cl(^-)</th>
<th>(\text{CCl}_3/\text{ClA}^-)</th>
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<tr>
<td>(\text{NH}_2^+)</td>
<td>0%</td>
<td>24%</td>
</tr>
<tr>
<td>(\text{OH}^+)</td>
<td>90%</td>
<td>5%</td>
</tr>
<tr>
<td>(\text{C}_3\text{H}_5^+)</td>
<td>0%</td>
<td>30%</td>
</tr>
<tr>
<td>(\text{O}^-)</td>
<td>0%</td>
<td>20%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(\text{CH}_3\text{O}^-)</td>
<td>60%</td>
<td>0%</td>
</tr>
<tr>
<td>(\text{EtO}^-)</td>
<td>45%</td>
<td>0%</td>
</tr>
<tr>
<td>(\text{F}^-)</td>
<td>95%</td>
<td>5%</td>
</tr>
<tr>
<td>(\text{HS}^-)</td>
<td>90%</td>
<td>10%</td>
</tr>
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<sup>a</sup> Including 5% Cl\(^2^-\).

Table 2. Comparison of the product ratio for reactions of the Chloromethanes.

<table>
<thead>
<tr>
<th>Reaction System</th>
<th>PT</th>
<th>(\Delta H_r) kcal/mol</th>
<th>Sn2</th>
<th>(\Delta H_r) kcal/mol</th>
</tr>
</thead>
</table>
| \(\text{NH}_2^+ + \text{CH}_3\text{Cl} \)
| 68% | -7.6 | 32% | -67 | 68% | -7.6 | 32% | -67 |
| \(\text{CH}_3\text{O}^- + \text{CH}_2\text{Cl}_2\)
| 90% | -6.0 | 10% | -49 | 90% | -6.0 | 10% | -49 |
| \(\text{CH}_3\text{COCH}_2^- + \text{CHCl}_3\)
| 100% | -12.0 | 0% | -48 | 100% | -12.0 | 0% | -48 |
| \(\text{CH}_3\text{S}^- + \text{CHCl}_3\)
| 100% | -0.7 | 0% | -33 | 100% | -0.7 | 0% | -33 |
| \(\text{OH}^- + \text{CCl}_4\)
| -  | - | 5%<sup>b</sup> | -61 | -  | - | 5%<sup>b</sup> | -61 |

<sup>a</sup> Data from S. Ingemann et al. J. Chem. Soc. Perkin II 1985, 837-840.<n<sup>b</sup> Major product is the adduct.

Figure 1

Figure 2
Tetranitromethane (TNM), a commercially available, stable liquid, has been shown to be very reactive towards electrons and reducing agents in the liquid phase. In addition, TNM is used in the condensed phase to label tyrosyl residues on proteins, to inactivate certain enzymes and has been examined as an oxidizer for liquid rocket propellants. We were led to examine the reactions of this intriguing compound for a number of reasons, including the wide-ranging utilization of the molecule and the paucity of mechanistic understanding of its reactions. We are always on the lookout for new chemical reagents that can be used to identify structural properties of gas-phase ions; the utilization of TNM to identify carbanionic intermediates in enzymatic chemistry suggested that TNM might be useful in the gas-phase as well. We have therefore examined the thermally-equilibrated (300 K), gas-phase ion-molecule reactions of TNM with a large number of anions by means of the flowing afterglow technique.

All the reactions of anions with TNM studied in this work are fast; representative rate coefficients are shown in Table 1. Three different product ions have been observed for the various reactant ions that have been examined; \( \text{C(NO}_2\text{)}_3 \) is generally the most abundant product ion and is the sole product ion for many reactions. The second most common product ion is NO\(_2^+\); this primary product ion also undergoes a facile secondary reaction with TNM to yield additional \( \text{C(NO}_2\text{)}_3 \). Four of the reactant ions (F\(^-\), PhC=C\(^-\), CN\(^-\), and Cl\(^-\)) also produce various amounts of an ion of form \( \text{NuC(NO}_2\text{)}_2^-\).

A typical reaction scheme, that for the reaction of Cl\(^-\) with TNM is shown in the Figure. Similar schemes can be written for each reactant ion examined. Two competitive processes are necessary to account for the observations; the first-formed ion neutral complex \( [\text{Cl}^- + \text{C(NO}_2\text{)}_3] \) can either undergo dissociative electron transfer followed by breakup to yield the major product ion, trinitromethide, or it can undergo an \( S_2^1 \) process to yield a product ion-neutral complex of chlorotrinitromethane and NO\(_2^-\). This latter product ion-neutral complex can simply dissociate to give the observed NO\(_2^-\) or it can undergo a second dissociative electron transfer followed by complex dissociation to give the chlorodinitromethane anion. Independent generation of NO\(_2^-\) and examination of its reactions with tetranitromethane confirm the secondary reaction indicated in the Figure. All reactions postulated in

<table>
<thead>
<tr>
<th>HA(^-)</th>
<th>( \Delta H^\circ_{\text{acid}} ) (kcal mol(^{-1}))</th>
<th>EA (eV)</th>
<th>( k_{\text{obs}} ) (cm(^3) molecule(^{-1}) s(^{-1}))</th>
<th>EFF (^a) (k_{\text{obs}}/k_{\text{coll}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>HO(^-)</td>
<td>390.8</td>
<td>1.83</td>
<td>2.57 (± 12%) \times 10^{-9}</td>
<td>1.11</td>
</tr>
<tr>
<td>H(_2)C=\text{C-OCCH}_3</td>
<td>371.9</td>
<td>1.80</td>
<td>1.33 (± 5%) \times 10^{-9}</td>
<td>1.06</td>
</tr>
<tr>
<td>H(_2)C=\text{C-CH}_3</td>
<td>369.1</td>
<td>1.76</td>
<td>1.42 (± 2%) \times 10^{-9}</td>
<td>1.03</td>
</tr>
<tr>
<td>NO(_2^-)</td>
<td>340.3</td>
<td>2.27</td>
<td>1.30 (± 10%) \times 10^{-9}</td>
<td>0.87</td>
</tr>
</tbody>
</table>
the Figure are thermochemically allowed and are consistent with all relevant observations and literature data. Two processes that might have been included in the Figure have been omitted. While there are reports that tetranitromethane does have a bound molecular radical anion, under the conditions of our room-temperature swarm experiment, we never found a trace of that species; hence the molecular radical anion of TNM, as either a distinct product or even as a postulated reaction intermediate has been omitted from the scheme. The second mechanistic omission is a pathway to produce the trinitromethide anion by way of an addition-elimination process; addition of the nucleophile to the nitrogen to form a tetravalent nitrogen adduct followed by elimination of \( ^1\text{C}(\text{NO}_2)_3 \). This latter pathway, while not formally excluded from occurring has no precedent and is not necessary to account for the structure-reactivity trends observed.

\[
\begin{align*}
[\text{Cl}^- + \text{C(NO}_2)_4] & \xrightarrow{\text{det}} [\text{Cl}^- + ^1\text{C}(\text{NO}_2)_3 + \text{NO}_2^-] \\
& \xrightarrow{\text{S}^2} [\text{ClC}(\text{NO}_2)_3 + \text{NO}_2^-] \\
& \xrightarrow{\text{m}z 46} [\text{ClC}(\text{NO}_2)_3^- + \text{NO}_2^-] \\
& \xrightarrow{\text{m}z 139} \text{ClC}(\text{NO}_2)_3^- + \text{NO}_2^- + \text{NO}_2^- \\
\end{align*}
\]

The structure-reactivity trends observed can be summarized as follows. All weakly nucleophilic species (HgCsCH-CHa", I", etc.) give exclusively trinitromethide anion. The excellent nucleophiles but which have moderate electron affinities, (H2N", MeO", etc.) give trinitromethide as the major product but also yield some NO2". The good, sterically small, nucleophiles which also have strongly bound electrons give varying amounts of the third product, NuC(NO2)_2" as well (eg., 50% from F", 100% from CN", 9% from Cl"). The acidity of trinitromethane is below 327 kcal mol\(^{-1}\), making this molecule a very strong carbon acid. Tetranitromethane also appears to be an efficient "plasma modifier", since it is able to convert free electrons and many common anions into very unreactive inert anions, (i.e., either di- or trinitrosubstituted methide). Tetranitromethane does not appear to be a useful probe for gas-phase anion structure since all types of anions give essentially the same products. Other experiments have also shown that \(^1\text{C}(\text{NO}_2)_3\) clusters to typical organic molecules only slowly in the flowing afterglow (0.30 Torr helium environment), hence even though trinitromethide anion can be produced in copious amounts, it does not appear to be a great candidate for a NICIMS clustering-type reagent ion.

Acknowledgment: This work has been supported by the National Science Foundation through a Presidential Young Investigator Award to J. G.
The synthesis of ammonia from $N_2$ and $H_2$ over an iron-oxide catalyst (Haber process) is an important industrial reaction. This process proceeds by

$$N_2 + 3H_2 \rightleftharpoons 2H_3 \quad (1)$$

initial dissociation of $N_2$ on the catalyst followed by a complex sequence of events leading to ammonia synthesis. Important species include surface bound $N$, $NH$, $NH_2$, $N_2H$, and $N_2H_2$. The chemistry of these intermediates is not well understood. We have applied Fourier transform mass spectrometry (FTMS) to generate intermediates of relevance to ammonia synthesis and subsequently characterized their properties (i.e., structure, rearrangements, etc.).

We have found a convenient route to the generation of $MM'\text{ONH}^+$ and $MM'\text{N}_2\text{H}_2^+$ ions ($M, M' = \text{Fe, Co, Ni}$) and have focused our attention on the $MM'\text{N}_2\text{H}_2^+$ species.

The $MM'\text{N}_2\text{H}_2^+$ are generated by sequential reaction of the corresponding dioxide with ammonia, process 2. There are several possible structures for $\text{MM'N}_2\text{H}_2^+$, structures 1-4. The structure of the $MM'\text{N}_2\text{H}_2^+$ ions was probed by monitoring isotopic exchange reactions with both N-ammonia and deuterated ammonia. In addition, collision activated dissociation (CAD) was also informative.

The specific ions studied include $\text{FeCoN}_2\text{H}_2^+$, $\text{FeNiN}_2\text{H}_2^+$, $\text{CoNiN}_2\text{H}_2^+$, and $\text{Co}_2\text{N}_2\text{H}_2^+$. All four ions undergo a single facile nitrogen atom exchange with $15N$-ammonia, process 3. This clearly indicates two distinct and non-interconverting types of nitrogen atoms. In addition, all four ions undergo exchange of both hydrogen atoms with $\text{ND}_3$ indicating that both hydrogens are chemically equivalent.

CAD of the $MM'\text{N}_2\text{H}_2^+$ yield three primary fragmentation, reactions 4-6, with elimination of $\text{N}_2\text{H}_2$ the dominant channel. $\text{FeCoN}_2\text{H}_2^+$ yields exclusively
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

\[ \text{process 6, FeN}_2\text{H}_2^+ \text{ and Co}_2\text{N}_2\text{H}_2^+ \text{ processes 4 and 6, and CoN}_2\text{H}_2^+ \text{ processes 4, 5, and 6.} \]

\[ \text{CAD of the product of reaction 3 yields exclusive loss of the} \]

\[ \text{N label for process 5.} \]

The isotopic exchange results combined with the CAD results are consistent with a nitrido-amido structure, for all four MM'N\text{H}_2^+ ions. The elimination of N\text{H}_2, presumably diazene, requires significant rearrangement prior to fragmentation.
A growing body of synthetic methodology has been developed in the last two to three decades which involves the use of stoichiometric or catalytic amounts of transition metals to mediate carbon-carbon bond forming reactions. Palladium-catalyzed cross-coupling reactions have become increasingly useful for the mediation of reactions of electrophiles which have sp or sp² carbons at or immediately adjacent to the electrophilic center. During a program directed toward the development of Group 10 metal complexes which would catalyze carbon-carbon bond formation using primary neopentyl electrophiles, we were attracted to a recent report claiming that dichloro-1,1'-bis(diphenylphosphino)ferrocenylpalladium(II) (dppf/PdCl₂) will effectively catalyze the cross-coupling of alkyl halides with Grignard reagents [2]. It was noted that the reaction required pre-reduction of dppf/PdCl₂ to dppf/Pd(0) using dialkylaluminum hydride (DIBAL) [2,5].

On attempting to apply this methodology to the cross-coupling of neopentyl halides, we discovered that treatment of any alkyl halide with Grignard reagents in the presence of either dppf/PdCl₂ or dppf/Pd(0) afforded only the products of reduction [3]. Since reduction would not have been expected whether or not cross-coupling occurred, we undertook a mechanistic study of the reduction [4]. It was determined that the palladium catalyst is not involved in the reduction when highly polar solvents, such as tetrahydrofuran are used [4]. In this case it was possible to elucidate two competing mechanisms both of which lead to reduction.

In less polar solvents, such as diethyl ether, reduction of the alkyl halide by the Grignard reagent does not occur in the absence of palladium catalysts. We have proposed that in these cases reduction occurs following a catalytic cycle common to many cross-coupling reactions. Thus, as shown in Scheme 1, we suggested that dppf/PdCl₂ is reduced by the Grignard reagent to dppf/Pd(0) [4,5], which then oxidatively adds the electrophile. Hydride transfer from the Grignard reagent to the resulting palladium(II) complex generates an olefin (from the Grignard reagent), magnesium halide, and an organopalladium(II) hydride. Reductive elimination from the palladium hydride should rapidly afford the observed alkanes with concomitant regeneration of the dppf/Pd(0) catalyst. The order of the oxidative addition and hydride transfer steps remains unclear.

Scheme 1. Proposed mechanism for the palladium-catalyzed reduction of alkyl halides to the corresponding alkanes.
Central to this proposal is the oxidative addition of the alkyl halide. However, oxidative addition of "unactivated" alkyl halides, those not containing sp or sp$^2$ carbons at or immediately adjacent to the electrophilic center, is unprecedented.

In recent years, we have shown that Group 10 metal complexes are readily analyzed using fast atom bombardment (FAB) mass spectrometry [6-8]. In this paper, we report on the analysis of the palladium-mediated Grignard reduction of alkyl halides of the type phenylpropyl iodide, phenylneopentyl iodide, neopentyl iodide, and methyl iodide to their corresponding alkanes. In general 2 to 2.5 equivalents of Grignard reagent were added to the palladium(II) pre-catalyst for reduction to palladium(0) after which a large excess of alkyl iodide was added to observe the product of oxidative addition as the first intermediate of the proposed reaction mechanism.

FAB mass spectra of the reaction mixtures were obtained by placing a 2 μl aliquot of reaction mixture on a standard FAB probe tip using sulfolane as the liquid matrix. When methyl iodide is used as the alkyl halide for this reaction, a molecular ion from the product of oxidative addition is observed at m/z 603 [9], which is assigned as the [M + H$^+$]$^+$ ion of the oxidative addition product shown in Scheme 1 (compound II; Figure 1). In addition, structurally significant fragment ions are observed. For example, ions observed at m/z 787 and 675 are assigned as [dppf/Pd] and [dppfPd(C$_2$H$_5$)]$^+$, respectively. In addition, ions at m/z 660 (with a 661 component in the isotope pattern) and 583 are assigned as (dppfPd(0))$^+$ and (dppfPd(0) - phenyl)$^+$, respectively. This is the first clear, unambiguous, observation of the intermediate, formed by oxidative addition, for unactivated alkyl halides.

Results from the phenylpropyl-, phenylneopentyl-, and neopentyl iodides was not as conclusive. In each case ions at m/z 787 ([dppfPd]$, 741 ([dppfPdI]$^+$), 689 ([dppfPd(Et)]$^+$), 660 ([dppfPd(0)]$^+$), and 583 ([dppfPd - phenyl]$^+$) were observed. Ions corresponding to an alkyl group containing palladium(0) catalyst (with or without iodide) were not observed. It can only be postulated that the organo-palladium bond is weak or that the palladium iodide species observed is formed by palladium(0) catalyst acting as a scavenger of iodide from solution.

![Figure 1. FAB mass spectrum of (dppf)PdCl$_2$ + 2.1 equivalents of EtMgBr + excess methyl iodide in sulfolane.](image)
BIRADICAL ISOMERS OF CYCLOPROPANOL AND OXETANE AND THEIR COUNTERPART IONS

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Department of Chemistry, The University of Akron, Akron, OH 44325-3601, U.S.A.

Biradicals, species with two radical centers in the same molecule, are the postulated intermediates of important thermal and photochemical reactions, including stereoisomerization, polymerization, and synthesis of ketones and alcohols. Biradicals are the neutral counterparts of distonic radical ions, which can be formed easily in the mass spectrometer via ion-molecule reactions or unimolecular rearrangement-fragmentations. Such distonic radical ions can then be used as a source of gaseous elusive biradicals in neutralization-reionization mass spectrometry (NRMS) experiments. In this study we produced and studied in the gas phase CH$_2$CH$_2$CHOH and CH$_2$CH$_2$OCH$_2$-, the ring-opened forms of cyclopropanol and oxetane, respectively.

-CH$_2$CH$_2$OCH$_2$- This 1,4-biradical is formed by neutralization of the distonic ion CH$_2$CH$_2$OCH$_2$+ which has been characterized as a stable cation by Grützmacher et al. Its CAD spectrum (Figure 1) is distinctively different from that of ionized oxetane, displaying no appreciable m/z 36-39 (C$_3$H$_n$+) fragments.

In the neutralization-reionization (NR) spectrum of •CH$_2$CH$_2$OCH$_2$ a recovered parent ion is observed indicating that the 1,4-biradical CH$_2$CH$_2$OCH$_2$+ survives, at least partly, intact. The absence of m/z 36-39 peaks proves further that ring-closure to more stable oxetane has not taken place in the μs time window of our NR experiment. The smaller molecular ion in the biradical spectrum (Figure 1) is consistent with a more severe fragmentation of the biradical into CH$_2$CH$_2$OCH$_2$+ which after reionization contribute to the intense m/z 24-30 peaks.

CH$_2$CH$_2$CH•OH An ideal precursor for this 1,3-biradical is the distonic ion CH$_2$CH$_2$CH•OH which can be produced from ionized 4-oxobutanoic acid and is a distinct C$_3$H$_6$O+ isomer according to McAdoo et al. Although the CAD spectrum of this ion (Figure 2) looks similar to the CAD spectrum of cyclopropanol+, small but significant differences, e.g., in the abundances of the m/z 57 (H-loss), 44,

1572
(CH₂ loss), and 43 (CH₃ loss) indicate that these two ions do not interconvert freely and must therefore be separated by a considerable barrier. The CAD spectrum of the allyl alcohol ion (not shown), on the other hand, is virtually identical to that of cyclopropanol‡, showing that the former readily rearranges to the latter which is the thermodynamically more stable isomer, in agreement with the photoelectron-photon coincidence studies of Bombach et al. Finally, the CAD spectra of ionized propanal and its enol are clearly different from the spectra of the distonic CH₂CH₂CH⁺OH ion and the cyclopropanol ion. Thus four C₅H₁₀O⁺ ions, namely CH₃CH₂CH=O⁺, CH₂=CHCH(OH)⁺, cyclopropanol⁺, and CH₂=CHCH⁺OH, represent stable, distinguishable isomers from which the counterneutrals can be prepared by neutralization. The corresponding NR spectra were measured in order to determine the fate of the 1,3-biradical CH₂CH₂CH⁺OH: is it stable, does it cyclize to cyclopropanol, or rearrange via H migration to propanal, the most stable of these C₅H₁₀ neutrals?

The appearance of a sizable recovered parent cation (m/z 58) in the NR of CH₂CH₂CH⁺OH (Figure 2) provides evidence that CH₂CH₂CH⁺OH can survive undissociated. The NR spectrum for this biradical is substantially different from that for propanal indicating that the isomerization CH₂CH₂CH⁺OH → CH₂CH₂CH=O does not occur. Furthermore, the structurally diagnostic peaks at m/z 43 and 44 (vide infra) are more intense in the biradical spectrum than in the spectrum for cyclopropanol showing that there is a noticeable barrier for CH₂CH₂CH⁺OH ring-closure to cyclopropanol. Consequently, the "Norrish like" biradical CH₂CH₂CH⁺OH is a chemically bound species.

**Figure 2.** (a,b,c,d) Partial CAD, O₂ and (e,f,g,h) partial +NR⁺, Xe/O₂, spectra of (a,e) CH₃CH₂CH=O⁺, (b,f) CH₂=CHCH(OH)⁺, (c,g) cyclopropanol⁺, and (d,h) CH₂CH₂CH⁺OH.
The unimolecular dissociation of dimethyl sulfide was investigated using threshold photoelectron photoion coincidence (TPEPICO) and tandem (MS/MS) mass spectrometry. From the TPEPICO study the breakdown graph is obtained in the energy range of 10.35 eV to 14.0 eV. Fig. 1 presents the TPEPICO breakdown diagram constructed from ion abundances obtained from peak areas in coincidence TOF mass spectra. The peaks of $\text{CH}_3\text{S}^*$ (m/z 47), $\text{CH}_2\text{S}^*$ (m/z 46) and $\text{CHS}^*$ (m/z 45) in TOF spectra were treated as one peak of m/z 47 since it was not possible to deconvolute these overlapping peaks reliably. Table I. presents the appearance potentials observed in the present work.

Table I. Appearance Potential (AP in eV)

<table>
<thead>
<tr>
<th>IONS</th>
<th>(m/z)</th>
<th>AP (This work)</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CH}_3\text{SCH}_2^*$</td>
<td>62</td>
<td>8.96</td>
<td>8.69c</td>
</tr>
<tr>
<td>$\text{CH}_3\text{SCH}_2^*$</td>
<td>61</td>
<td>10.66b</td>
<td>10.85d</td>
</tr>
<tr>
<td>$\text{CH}_2\text{S}^*$</td>
<td>47</td>
<td>10.5b</td>
<td>10.8d</td>
</tr>
<tr>
<td>$\text{CHS}^*$</td>
<td>46</td>
<td>10.5c</td>
<td></td>
</tr>
<tr>
<td>$\text{CHS}^*$</td>
<td>45</td>
<td>14.0d</td>
<td></td>
</tr>
</tbody>
</table>

* Metastable appearance energy measured using a Kratos MS 50 TA
b Obtained by taking the mid point of two energies: last absent and first observed
c Ref. 2
d Ref. 2

The breakdown diagram is dominated by ions of mass m/z 47 (46, 45) throughout the energy range covered. The relative abundance of m/z 61 increases from about 12.09 eV to 13.54 eV whereas the relative abundance of ion m/z 47 (46,45) decreases in the same energy range. In the He-I photoelectron spectrum$^1$ of $\text{CH}_3\text{SCH}_2^*$ this energy region corresponds to the $B^2B_2$ ionization transition state of the molecular ion. Ionization to this state leads to a complete dissociation of the molecular ion. Our breakdown diagram suggests that the ionization transition to the $B^2B_2$ state produces predominantly $\text{CH}_3\text{S}^*$ (m/z 47, 46, 45). The decrease in the relative abundance of m/z 61 ion around 13.54 eV is accompanied by a rise in $\text{SH}^*$ (m/z 35) (not shown in Fig 1) and $\text{CHS}^*$ (m/z 47,46,45) ions. In the He-I photoelectron spectrum this energy region is characterized by a broad band which includes the transition states of $\text{CH}_3\text{SCH}_2^*$ localized in the methyl group. Ionization to these transition states gives rise to the formation of $\text{SH}^*$ and $\text{CH}_3^*$ cations that appear at higher photon energies.

In earlier studies$^2$ it has been observed that the fall in the yield of $\text{CH}_3\text{SCH}_2^*$ is accompanied by the formation of $\text{CHS}^*$ and $\text{C}_2\text{H}_3^*$. This observation was
attributed to a successive decomposition of CH$_3$SCH$_2^*$ leading to the formation of CHS* or C$_2$H$_3^*$. Although successive dissociation cannot be ruled out, our CA MS/MS study has established that all major fragments originate directly from the parent ion (Fig. 2). In the present experiment the major ions observed are C$_2$SCH$_3^*$ (m/z 62), CH$_3$SCH$_2^*$ (m/z 61), CHS* (m/z 47), SH* (m/z 35) and C$_2$H$_3^*$ (m/z 27). Fig 3. presents the metastable ion spectrum of CH$_3$SCH$_3^*$ measured on a Kratos MS 50 TA.

From the breakdown curves, MS/MS study and energetics considerations the major fragmentation pathways are derived as shown below:

\[
\begin{align*}
\text{CH}_3\text{SCH}_3^* & \quad \rightarrow \text{CH}_3\text{SCH}_2^* + e \\
\text{CH}_3\text{SCH}_2^* & \quad \rightarrow \text{CH}_3\text{S}^* + \text{CH}_4 \\
\text{CH}_3\text{S}^* & \quad \rightarrow \text{CHS}^* + \text{CH}_4 + \text{H} \\
\text{CHS}^* & \quad \rightarrow \text{SH}^* + \text{C}_2\text{H}_3 + \text{H}_2 \\
\text{C}_2\text{H}_3^* & \quad \rightarrow \text{C}_2\text{H}_3 + \text{H}_2 \text{S} + \text{H}
\end{align*}
\]

Our MNDO calculation agree with earlier reports that the stable structures of fragments of mass 47 and 46 are CH$_3$SH* and CH$_3$S* respectively. The stable structure of the fragment ion CH$_3$S* has long been a subject of interest. Early collisional activation study of dimethyl sulfide has revealed that at low ionization electron energies the ion of mass 47 produced is the mercaptomethyl (CH$_3$SH*) cation and not the thiomethoxy cation (CH$_3$S*). In addition, ab initio calculations have shown that the singlet mercaptomethyl cation is the most stable isomer lying about 113 KJmol$^{-1}$ lower in energy than the triplet thiomethoxy cation.

References
Production mechanisms of quasi-molecular ions in atmospheric pressure spray (APS) ionization

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*Nagaoka University of Technology, Nagaoka, Niigata 940-21, Japan

In spray ionization methods, mechanisms by which quasi-molecular ions are produced have not yet been investigated in detail. For example, the ion evaporation model proposed by Iribarne and Thomson\(^1\) is often accepted as the mechanism producing quasi-molecular ions by electrospray and thermospray, but this model has not been proved experimentally. Ion-molecule reactions in the gas phase can also contribute to the final production of quasi-molecular ions. It is therefore important to investigate their roles in the spray ionization generation of quasi-molecular ions. Recently, we have developed another spray ionization method, called atmospheric pressure spray (APS) ionization.\(^2\) The present work uses APS to distinguish a gas-phase ion-molecule reaction from ion evaporation in producing quasi-molecular ions.

Positive ions produced in APS ionization from sucrose, ammonia, and alanine in aqueous solutions have been measured with a double focusing mass spectrometer, and the obtained mass spectra are analyzed as a function of the distance between the nozzle tip and the mass-spectrometer sampling aperture. The time for an ion-molecule reaction at atmospheric pressure increases with this distance.

Cationized sucrose molecules, i.e., \(\text{MNa}^+\), are produced from an aqueous solution containing \(7.5 \times 10^{-5}\) mol/l of sucrose and \(4.0 \times 10^{-7}\) mol/l of NaOH. As shown in Fig. 1, the relative intensity of \(\text{MNa}^+\) ion is independent of the distance. It is therefore concluded that cationized sucrose molecules are produced by the spray process rather than by a cation transfer reaction in the gas phase. This spray formation of \(\text{MNa}^+\) may thus be described in terms of the ion evaporation model\(^1\) in which final ion formation is completed during the spray.

Figure 2 shows the relative ion intensities for a \(5.0 \times 10^{-6}\) mol/l solution of ammonia in deionized water as a function of the distance. The relative intensity of \(\text{NH}_4^+\) ions increases with a decrease in that of \(\text{H}_3\text{O}^+\). Furthermore, the \(\text{NH}_4^+\) intensity tends to be zero at zero distance. This indicates that the neutral \(\text{NH}_3\) molecules are evaporated from the ammonia solution and that \(\text{NH}_4^+\) ions are produced in the gas phase by an ion-molecule reaction with \(\text{H}_3\text{O}^+\) produced by the spray.

Figure 3 shows the observation for \(5.0 \times 10^{-6}\) mol/l aqueous solution of alanine. The relative intensity of the protonated alanine molecule, \(\text{MH}^+\), increases with the distance, but tends to be about 50 % at zero distance. This indicates that neutral as well as protonated...
alanine molecules are produced by the spray, and that the former species are partly protonated after the spray by a similar ion-molecule reaction in the gas phase. (Since the atmosphere in the ion source was contaminated by ammonia in this particular experiment, a small amount of $\text{NH}_4^+$, not shown in the figure, was also observed as a result of an ion-molecule reaction with $\text{H}_3\text{O}^+$) Therefore, it is concluded that the production mechanism of quasi-molecular ions depends on the molecular species.

References
Ammonia Laser Desorption/Chemical Ionization with Ammonium Bromide: Fourier Transform Ion Cyclotron Resonance Mass Spectrometry of Aromatic Hydrocarbons

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Laser desorption/ionization combined with Fourier transform ion cyclotron resonance mass spectrometry (LD/FT/ICR/MS) is a proven technique for the analysis of nonvolatile materials. Unfortunately, LD tends to produce a large excess of neutrals relative to ions. Because of the inherent inefficiency of ion formation in LD, several methods have been applied in FT/ICR/MS to increase the number of ions formed after desorption. These include adding a small amount of an alkali halide salt (e.g. KBr) to the sample [1] and post-desorption methods such as electron ionization [2], resonance enhanced multiphoton ionization (REMPI) [3] and chemical ionization (CI) with an added reagent gas [4].

In this poster, we report that ammonia LD/CI can be performed simply by addition of a small amount of ammonium bromide (NH₄Br) to an involatile sample, i.e. the ammonium salt is used in place of ammonia reagent gas. Such an experiment does not require addition (e.g., by pulsed valve) of reagent gas from outside the vacuum chamber, and should thus prove useful for applications in which high mass resolution is needed. For various aromatic hydrocarbons, (M+H)⁺ ions are produced in enhanced abundance as a result of chemical ionization. The mechanism proposed for ammonia LD/CI with NH₄Br is given as follows:

\[
\begin{align*}
\text{M}^\text{sol} & \rightarrow \text{M}^\text{as} + \text{NH}_4^+ + \text{Br}^- \\
\text{M}^\text{as} + \text{NH}_4^+ & \rightarrow [\text{M}^\text{as} - \text{H} - \text{NH}_3]^+ + \text{NH}_3 \text{ gas} \\
[\text{M}^\text{as} - \text{H} - \text{NH}_3]^+ + \text{X} & \rightarrow \text{MNH}_4^+ + \text{X} \text{ gas}
\end{align*}
\]

In which M is the analyte and X denotes any neutral species which may be in the ion trap. Proton transfer (Eq. 4a) will occur only if the proton affinity of the sample exceeds that of ammonia. Thus, the LD/CI technique should prove to be chemically selective. Cation attachment (Eq. 4b) is unlikely because the FT/ICR/MS operating pressure is too low to collision-stabilize the intermediate.

Results:

We have observed some degree of protonation with this alternative LD/CI technique for the hydrocarbons: pyrene, 2-methylanthracene, hexamethylbenzene, and coronene. Only for hexamethylbenzene was [M + H⁺] more abundant than M⁺, as shown in Figure 1. For coronene, the most abundant species in the LD/FT/ICR mass spectrum is the unusual protonated fragment ion, C₁₂H⁺, which is formed when coronene is protonated, followed by loss of six C₆H₂ molecules located at the perimeter of the coronene ring system, as shown in the following mechanism:
The ratio, \((\text{MH}^+)/\text{(M}^+)\), is controlled by at least three factors: laser irradiance, concentration of \(\text{NH}_4^+\) in the plume of desorbed species, and fragmentation of \(\text{MH}^+\) to form \(\text{M}^+\). Thus, the \((\text{MH}^+)/\text{(M}^+)\) ratio should not be used to compare the relative efficiencies of LD/CI and photolization, since proton transfer at a collision-limited rate is expected to occur whenever the proton affinity of the analyte molecule significantly exceeds the proton affinity of the reagent [5]. A fuller account of this work will be published elsewhere [6].

\[
\begin{align*}
\text{C}_{24}\text{H}_{12}^+ + \text{NH}_4^+ & \rightarrow \text{C}_{24}\text{H}_{13}^{++} + \text{NH}_3 & (5a) \\
\text{C}_{24}\text{H}_{13}^{++} & \rightarrow \text{C}_{12}\text{H}^+ + 6\text{C}_2\text{H}_2 & (5b)
\end{align*}
\]

Figure 1. LD/FT/ICR mass spectrum of hexamethylbenzene with added ammonium bromide.

References

AN INVESTIGATION OF THE MECHANISM OF GLYCOSIDIC BOND CLEAVAGE IN THE 
NH3-DCI MASS SPECTRA OF PERACETYLATED CARBOHYDRATES: *J. M. Pelletier, R. W. 
Smith, D. B. MacLean, Department of Chemistry, McMaster University, Hamilton, Ontario, L8S 4M1, 
Canada and W. A. Szarek, Department of Chemistry, Queen's University, Kingston, Ontario, K7L 
3N6, Canada.

The NH3-DCI mass spectra of peracetylated carbohydrates exhibit ions which correspond to 
species resulting from cleavage on either side of a glycosidic oxygen with attachment of (H + NH4\(^+\) or 
(CH\(_2\)CO + NH4\(^+\)) to the observed fragment. The first of these two ion types will be referred to as an 
H transfer ion and the second will be referred to as an acyl transfer ion. Both types of ion can be used 
to determine the sequence of carbohydrate residues in an oligosaccharide and thus are useful in the 
structure elucidation of carbohydrates. However, little has been reported regarding the structure and 
formation of these ions, although Dougherty et al. have proposed that the acyl transfer ions arise by 
"thermolysis of the parent molecule followed by NH4\(^+\) attachment to the neutral fragments".1

In an attempt to gain further insight into the structure of the ions formed and the processes 
occurring on glycosidic bond cleavage, the spectra of the gentiobioses shown below have been studied.

Mass spectra were obtained on a VG Analytical ZAB-E mass spectrometer. The electron beam energy 
was ~ 70 eV. The source temperature was maintained at 200°C; the DCI probe was not heated. The 
reagent gas (Matheson, anhydrous ammonia) was maintained at a pressure that resulted in a 20:1 ratio 
of NH4\(^+\) to (N\(_2\)H\(_7\))\(^+\). Mass-analysed ion kinetic energy (MIKE) spectra were acquired employing the 
CI conditions described above.

Octa-O-acetylgentiobiose (1) was obtained from Sigma Chemical Company. The synthesis of 
compounds 2 to 5 will be reported elsewhere. The purity and structure of the compounds studied was 
established by \(^1\)H-NMR, gas chromatography and mass spectrometry. Compound 3 was found to be a 
60:40 mixture of anomers as determined by \(^1\)H-NMR. The two anomers were separated in a GC-MS 
experiment, which showed that their spectra were essentially the same. A J&W Scientific DB 
1701-30W column was used at 280°C isothermal with a head pressure of 1.5 psi and with helium as 
the carrier gas.

The H transfer ions derived by cleavage at \(x\) or \(y\) in compound 1 both appear at m/z 366 and 
cannot be distinguished from each other. In the case of compounds 2 and 3 the H transfer ions derived 
from the two ends of the molecule can be distinguished and are represented as shown below. MIKE 
experiments indicate that the H transfer ions are not derived direct from the [M+NH4\(^+\)]\(^+\) ions of 1 - 3.

The MIKE spectra of ions 2b and 3a were compared with the MIKE spectra of the 
pseudomolecular ions, [M+NH4\(^+\)]\(^+\), of 4 and 5, respectively.

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The MIKE spectra of ions 2b and [4 + NH₄]⁺ were virtually identical, as were the MIKE spectra of ions 3a and [5 + NH₄]⁺, which suggests that the H transfer ions have the structures indicated.

The ratio of intensities of 2a:2b and 3a:3b are 4:1 and 3:1, respectively. The fact that ions of type a and b are observed confirms that cleavage occurs on either side of the glycosidic oxygen. However, the relative ratios of the ions indicate that cleavage at 'x' dominates in gentiobiose.

Similarly, acyl transfer ions can be formed by cleavage at 'x' or 'y'. The two ions generated from compound 1 will both appear at m/z 408 and thus, cannot be distinguished from each other. In the case of compounds 2 and 3 it is possible to determine the end of the disaccharide from which the ion is derived and to determine the type of the acyl group transferred.

In the spectrum of compound 2 only one acyl transfer ion at m/z 420 is observed, while in the spectrum of compound 3 acyl transfer ions at m/z 408, m/z 411, and m/z 420 are observed. The acyl transfer ions are assigned the structures shown below.

![Diagram](image)

The MIKE spectra of ion 3c and the [M + NH₄]⁺ ion of peracetylated glucose are the same, which suggests that the structure of the two ions is the same. Both of their MIKE spectra are characterized by a single intense ion resulting from the loss of NH₄⁺ and the group at C-1. This type of fragmentation has been observed previously. The MIKE spectrum of ion 2d also exhibits a single intense ion, which indicates that a nondeuterated acetate was lost from C-1. The position of the nondeuterated acetate group in ion 2d suggests that acyl transfer ions are formed with selective transfer of an acyl group to C-1 in the case of those ions derived from the non-reducing end of the disaccharide. Attempts to obtain MIKE spectra of ions 3d and 3e were unsuccessful due to the low intensity of those ions.

Ion 3e at m/z 408 and ion 3d at m/z 411 differ by three mass units. This difference can occur only if the acyl transfer ions in question are formed by transfer of an intact acyl group. If these ions were formed by the addition of ketene to the free hydroxyl of a H transfer ion then addition of dideuteroketene instead of ketene would cause a two mass-unit shift.

Finally, both the sugar moiety and the transferred acyl group in ion 3c are derived from the non-reducing end of the disaccharide. Since the reducing end of compound 3 contains only trideuteracetacetyl groups, the transferred acyl group in ion 3c must have come from a different molecule.

Our experiments did not provide any insight into the nature of the species from which the H transfer ions and the acyl transfer ions are derived. However evidence was found to support the previously proposed structures for these ions. Furthermore, in the case of the acyl transfer ions there is evidence to suggest that they are formed, at least in part, by intermolecular transfer of an acyl group and by transfer of that acyl group to C-1 for ions derived from the non-reducing end of the disaccharide. Finally the acyl transfer ions appear not to be formed by addition of ketene to a free hydroxyl.

Effect of Substituent Identity on Electron Capture Enhancement in Negative Chemical Ionization of Pentafluorobenzoyl Derivatives of Substituted Phenylpiperazines

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Introduction:

We have been interested in the analysis of phenylpiperazine compounds, since they are metabolites of a number of CNS drugs. GC/MS is the analytical method of choice because it can provide sufficient sensitivity to quantitate at the low levels of metabolites produced by the typical dosings of these drugs. Derivatization at the unsubstituted nitrogen of the piperazine ring by pentafluorobenzoyl chloride (PFB) was utilized to allow these compounds to elute from the GC column.

GC-EI/MS analysis yielded spectra containing relatively intense ions but with significant fragmentation at the derivatization site as shown in Fig. 1 for the 3-chloro analog. Similar spectra were obtained for all the compounds studied. Given their electron capture capability, it was not surprising that the derivatized phenylpiperazine analogs produced negative chemical ion (NCI) spectra containing only molecular ions at intensities far greater than the electron impact spectra. The degree of intensity enhancement was not the same for all analogs, however; it appears to depend on not only the identity of the substituent but also its position on the ring. The compounds shown in Table I were studied at three different source temperatures: 100°, 150°, and 200°C (ammonia gas pressure optimized at each temperature). Ion yields were compared by substituent, position and source temperature:

Table I Compounds Studied

<table>
<thead>
<tr>
<th>X = H, 2-Cl, 3-Cl, 4-Cl,</th>
</tr>
</thead>
<tbody>
<tr>
<td>= 4-F, 2-CH₃, 2-OCH₃, 3-CF₃</td>
</tr>
</tbody>
</table>

The following are spectra of 1-(3-chloro)phenylpiperazine demonstrating significant fragmentation at the derivatization site in the molecule (PFB M.W. = 195 amu) under EI ionization.

Fig. 1 Spectra of 1-(3-chloro)phenylpiperazine

EI, source 200°C

NCI, source 200°C
Table II summarizes the integrated ion intensities from 300 to 450 amu for each of the compounds in counts x 10^5.

<table>
<thead>
<tr>
<th>Analog of Phenylpiperazine</th>
<th>Source Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100°C</td>
</tr>
<tr>
<td>2-methoxy</td>
<td>13</td>
</tr>
<tr>
<td>2-methyl</td>
<td>18</td>
</tr>
<tr>
<td>unsubstituted</td>
<td>13</td>
</tr>
<tr>
<td>4-chloro</td>
<td>12</td>
</tr>
<tr>
<td>2-chloro</td>
<td>18</td>
</tr>
<tr>
<td>3-chloro</td>
<td>17</td>
</tr>
<tr>
<td>4-fluoro</td>
<td>37</td>
</tr>
<tr>
<td>1-(1,1,1-trifluoro-3-methyl)</td>
<td>30</td>
</tr>
</tbody>
</table>

**Results:**

1. Source temperature had some effect on the relative resonant electron capture ability of these analogs but no consistent trend was observed.

2. Relative to the unsubstituted phenylpiperazine, electronegative substituents enhanced electron capture provided while alkyl substituents diminished it.

3. Position of the substituent on the phenyl ring was significant in the case of the chlorinated positional isomers, i.e., 3-chloro was slightly more intense than the 2-chloro analog at the two higher source temperatures and twice as intense as the 4-chloro compound.

**Discussion:**

The substituent effect on phenylpiperazine analogs relative to 1-phenylpiperazine is analogous to that of substituent groups on aniline. Electron donating groups such as -CH₃ and -OCH₃ stabilize a positive charge on the molecule through resonance. Electron withdrawing groups such as halogens and -CF₃ reduce the ability of the molecule to have a positive charge enhancing the likelihood of resonant electron capture. This results in greater intensity of the molecular ion largely independent of source temperature.

We find no obvious explanation for the magnitude of the differences in the electron capture efficiencies of positional isomers of the chlorinated phenylpiperazine analog. The meta position consistently showed the greatest response.
Use of Molecular Modelling in Interpretation of the Electron Capture Negative Chemical Ionization Mass Spectra of Polychlorinated Biphenyls.

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Electron capture negative chemical ionization (ECNCI) mass spectra of polychlorinated biphenyls (PCB) show fragmentation that is related to the molecular structure of the compounds. The fragmentation is influenced predominately by two structural characteristics. Fragmentation is reduced with increasing number of chlorine atoms on the PCB congener, but conversely becomes more pronounced as the number of chlorine atoms in the 2 & 6 (ortho) positions increases. Correlations between structure and ECNCI fragmentation have also been reported for polybrominated biphenyls (PBB) and polychlorodibenzo-p-dioxins (PCDD).

The importance of fragmentation differences in ECNCI spectra has been demonstrated by its use in the identification of a PCB congener in environmental samples that had not been previously detected because of its coelution with PCB congener IUPAC # 138.

Molecular model results for PCDD, using complete neglect of differential overlap (CNDO) approximations, have been evaluated with respect to ECNCI spectra. Fragmentation was related to the energies of the lowest unoccupied molecular orbital (LUMO) and LUMO+2 (where LUMO+n = molecular orbital n levels above the LUMO).

The objective of the present work was to evaluate ECNCI spectra of PCB congeners in terms of their molecular parameters derived from the MOPAC molecular model program which uses modified neglect of differential overlap (MNDO) approximations. Correlations between molecular structural properties and ECNCI spectra may result in better understanding of the ECNCI process and be useful for obtaining structural information on unknown compounds.

ECNCI mass spectra (moderator = methane) of 50 PCB congeners (tetras, pentas, hexas) were obtained on an Extrel ELQ 400-2 quadrupole mass spectrometer.

The molecular model program, MOPAC, was obtained from the Quantum Chemistry Program Exchange (QCPE, Indiana University, Bloomington, IN) and was run on a DEC Micro VAX II computer. MOPAC, using the AM1 Hamiltonian operator, was run for all 209 PCB congeners and biphenyl. Model generated molecular properties of particular interest were: heats of formation, twist angle between the phenyl rings, and the energies of the highest occupied molecular orbital (HOMO), LUMO, LUMO+1, and LUMO+2.

MOPAC results were related to various molecular structural properties. The calculated heats of formation decreased as the number of substituent chlorine atoms increased. The energies of the HOMO, LUMO, LUMO+1 and LUMO+2 were similarly reduced. The calculated twist angles increased as the number of 2 & 6 Cl atoms increased. Thus, for 0 - 4 Cl, the mean twist angles were 41°, 56°, 76°, 86° and 87°, respectively.

The number of 2 & 6 Cl atoms on a molecule particularly influenced the LUMO energy. The change in LUMO energy was observed as a decrease in the energy difference between the LUMO and LUMO+1. For 0 - 4 chlorine atoms, the mean differences were -0.49, -0.32, -0.16, -0.09, and -0.07 eV, respectively. The overall influence of the increasing number of
chlorine atoms on the congeners tended to disguise some of the relationships. However, within homologous groups of congeners, e.g. pentachlorobiphenyls, the effect of the number of 2 & 6 Cl atoms was clearly observable from plots of the number of chlorine atoms against twist angle, LUMO and LUMO - LUMO+1 energies.

ECNIC spectra of PCB included [M]', [M-H]', [M-Cl]' and [M-2Cl]' ions. Fragmentation ratios of [M]/[M-X]' (where X = H, Cl, 2Cl) were calculated and correlated with various MOPAC molecular parameters including HOMO, LUMO, LUMO+1, LUMO+2 energies, twist angle and the number of 2 & 6 Cl atoms. Correlations of these parameters with the [M]/[M-Cl]' fragmentation ratios were observed and when the fragmentation ratios were log, transformed the data yielded linear results. Because LUMO energy is related to both the twist angle and the number of 2 & 6 Cl atoms, log,([M]/[M-Cl]') is in turn related to the latter two parameters.

Fragmentation in ECNIC spectra of PCB congeners was found to be related to molecular parameter values calculated by the MOPAC program. The log,([M]/[M-H]') and log,([M]/[M-Cl]') fragmentation ratios were correlated with LUMO, twist angle and number of 2 & 6 Cl atoms. Correlation coefficients were higher for chlorine atom loss than for hydrogen atom loss.

The energies of the HOMO, LUMO, LUMO+1 and LUMO+2 orbitals all became increasingly negative as the number of chlorine atoms increased. This indicated that the PCB congeners with the greatest number of chlorine atoms have an increasing ability to stabilize an ionizing electron. This would explain the increased frequency of molecular ion occurrence in the spectra of PCB as the number of chlorine atoms increased. However, the LUMO energy increased in negativity to a lesser extent as the number of 2 & 6 Cl atoms increased. This provided a basis for explaining the observed greater PCB fragmentation in ECNIC spectra as the number of 2 & 6 Cl atoms increased. It is hypothesized that molecules exhibiting less difference between the LUMO and LUMO+1 energies are less able to stabilize the energy of an ionizing electron and therefore fragment.

The threshold values of the orbital energies that encourage fragmentation are currently being investigated. This is being done with respect to the energies anticipated to be present in the ECNIC process. This may provide additional information on the reaction processes involved in this type of ionization.

References
THE EFFECT OF EPOXIDE GROUPS ON THE ELECTRON CAPTURE NEGATIVE IONIZATION MASS SPECTROMETRIC RESPONSE OF STEROIDS AND RELATED COMPOUNDS. Helen K. Mayer1, William Reusch1, and J. Throck Watson1,2, Departments of Chemistry1 and Biochemistry2, Michigan State University, E. Lansing, MI 48824.

Under electron capture negative ionization (ECNI) conditions, certain combinations of double bonds, carbonyl groups, and halogens have been shown to either not affect, decrease, or greatly enhance the relative response of steroids and other cyclic compounds (1). The presence of an epoxide group also may be important for enhanced response.

All compounds were analyzed under similar conditions using a JEOL JMS-AX505H mass spectrometer coupled with a Hewlett Packard 5890 gas chromatograph. Methane was used as the reagent gas because it does not favor the ionization of any particular compound. The following instrumental conditions were used: source temperature, 200°C; pressure, 2 x 10⁻⁵ torr; and accelerating voltage, 3 keV. All compounds were analyzed first in the full scan mode to determine the major ions; then these ions were used for replicates analyses by selected ion monitoring.

For enhanced response, an epoxide group must be near an electrophilic group or alpha to a carbonyl (see Table 1). The position of this carbonyl group is not important; the electron-capture capacity is the same. In fact, an epoxide alpha to a carbonyl is much more electron-withdrawing than a comparable conjugated ene-one system. For example, A4-3,17-dione has a relative response of 1.2; however, if a 4,5-epoxide replaces the double bond, the relative response increases to 39. The stereochemistry of the epoxide group does not affect its response. In many cases, the other functional groups in the molecule have a limited effect on the overall response; for example, P-16α,17α-epoxy-3,20-dione and Pδ-16α,17α-epoxy-3,20-dione have almost the same response. The presence of an epoxy group in conjunction with a carbonyl must be the most important contribution to the overall response of the compound.

Electron affinities have been shown to correlate with reduction potentials (E1/2) in aprotic solvents (2). In many cases, the trend in E1/2 correlated with the ECNI relative response (see Table 1). However, a direct correlation is not always possible, as shown in the comparison of P-4α,5α-epoxy-3,20-dione, P-16α,17α-epoxy-3,20-dione, and C-4δ,5δ-epoxy-3-one. All three have the same E1/2, but only the pregnanes have a similar ECNI response. Other researchers have noted variations between electron affinity values and ECNI responses for a series of polyaromatic hydrocarbons (3).

It has been noted in a previous study (1) that the presence of a M⁺ ion rather than a [M-H]⁻ ion in the ECNI mass spectrum of a ketosteroid is indicative of a high-response compound. This is not necessarily true of steroid epoxides. Compounds that form M⁺ ions have relative responses ranging from 9 to 200; compounds that form [M-H]⁻ ion have relative responses overlapping this range.

Trichothecenes (Table 2) also have enhanced ECNI responses. The presence of an epoxide in deoxynivalenol acetate does not enhance its response in comparison to the unepoxylated DOM-1 acetate. In this case, the epoxide group is not near a carbonyl group; therefore, structural features other than the epoxide group must be responsible for this particular enhanced response.

**KEY**

A Androstane C19 structure
P Cholestane C27 structure
C Cholesterol C21 structure
α or β before a letter refers to the configuration at C-5 of steroids (e.g., αA for 5α-androstane)

Superscripted number after a letter denotes a double bond; for example, A4 = 4-androstene.

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Table 1: ECNI Relative Responses of Epoxysteroids

<table>
<thead>
<tr>
<th>Compound</th>
<th>Major Ions</th>
<th>Relative Response</th>
<th>(E_{1/2}) (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha)-A-3-one</td>
<td>([M-H]^+)</td>
<td>1.0 ± 0.06</td>
<td>**</td>
</tr>
<tr>
<td>(\alpha)-A-1(\alpha),2(\alpha)-epoxy-3-one</td>
<td>([M-H]^+)</td>
<td>2.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>(\alpha)-A-9(\alpha),11(\alpha)-epoxy-3-one</td>
<td>([M-H]^+)</td>
<td>5.0 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>A(^4)-3-one</td>
<td>([M-H]^+)</td>
<td>1.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>A(^4)-16(\alpha),17(\alpha)-epoxy-3-one</td>
<td>([M-H]^+)</td>
<td>1.8 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>(\alpha)-A-3-one-17-ol acetate</td>
<td>([M-H]^+)</td>
<td>1.8 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>A(^4)-5(\beta),5(\beta)-epoxy-3-one-17-ol acetate</td>
<td>([M-H]^+)</td>
<td>26 ± 1</td>
<td></td>
</tr>
<tr>
<td>A(^4)-4(\beta),5(\beta)-epoxy-3-one-17-ol acetate</td>
<td>([M-H]^+)</td>
<td>200 ± 18</td>
<td>-1.04,-2.05</td>
</tr>
<tr>
<td>A(^4)-4,5(\alpha)-epoxy-3-one-17-dione</td>
<td>([M-H]^+), ([M-CH_3]^+)</td>
<td>2.1 ± 0.1</td>
<td>-2.03</td>
</tr>
<tr>
<td>(\alpha)-A-3,17-dione</td>
<td>([M-H]^+)</td>
<td>1.0 ± 0.05</td>
<td>**</td>
</tr>
<tr>
<td>A(^4)-3,17-dione</td>
<td>([M-H]^+)</td>
<td>1.2 ± 0.1</td>
<td>-2.27</td>
</tr>
<tr>
<td>A(^4)-4(\beta),5(\alpha)-epoxy-3,17-dione</td>
<td>([M-H]^+)</td>
<td>39 ± 1</td>
<td></td>
</tr>
<tr>
<td>(\alpha)-P-3,20-dione</td>
<td>([M-H]^+)</td>
<td>1.2 ± 0.1</td>
<td>-2.23</td>
</tr>
<tr>
<td>P-4(\alpha),5(\alpha)-epoxy-3,20-dione</td>
<td>([M-H]^+)</td>
<td>21 ± 0.9</td>
<td>-2.41</td>
</tr>
<tr>
<td>(\alpha)-P-16(\alpha),17(\alpha)-epoxy-3,20-dione</td>
<td>([M-H]^+)</td>
<td>24 ± 0.2</td>
<td>-2.41</td>
</tr>
<tr>
<td>P(^4)-16(\alpha),17(\alpha)-epoxy-3,20-dione</td>
<td>([M-H]^+)</td>
<td>29 ± 3</td>
<td>-2.25</td>
</tr>
<tr>
<td>(\alpha)-P-16(\alpha),17(\alpha)-epoxy-3,11,20-trione</td>
<td>([M-H]^+)</td>
<td>9.1 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>(\alpha)-P-3,11,20-trione</td>
<td>([M-H]^+)</td>
<td>63 ± 2</td>
<td></td>
</tr>
<tr>
<td>C-5(\alpha),6(\alpha)-epoxy</td>
<td>([M-H]^+)</td>
<td>0.13 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>C-3-cyano-5(\alpha),6(\alpha)-epoxy</td>
<td>([M-H]^+), ([M-H]^+), ([M-H]^+)</td>
<td>6.8 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>C-3-CF(^3),5(\alpha),6(\alpha)-epoxy</td>
<td>([M-CF]^+), ([M-H]^+), ([M-H]^+)</td>
<td>6.4 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>C-5(\alpha),6(\alpha)-epoxy-4-one</td>
<td>([M-H]^+)</td>
<td>6.1 ± 0.1</td>
<td>-2.68</td>
</tr>
<tr>
<td>C-5(\beta),6(\beta)-epoxy-4-one</td>
<td>([M-H]^+)</td>
<td>5.2 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>C-3,5(\beta),5(\beta)-epoxy-3-one</td>
<td>([M-H]^+)</td>
<td>9.2 ± 1.2</td>
<td>-2.41</td>
</tr>
<tr>
<td>C(^4)-3,1(\alpha),2(\alpha)-epoxy-3-one</td>
<td>([M-H]^+)</td>
<td>58 ± 6</td>
<td>-1.50,-1.82</td>
</tr>
</tbody>
</table>

*A-3-one has been assigned a relative response of 1.0; n = 3-5.*

**The reduction potential is more negative than that of the solvent (\(E_{1/2} = -2.97\) V).

Table 2: ECNI Relative Responses of Mycotoxins and Trichotheccenes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Major Ions</th>
<th>Relative Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deoxynivalenol acetate</td>
<td>([193]^+), ([M-H]^+)</td>
<td>9.6 ± 0.8</td>
</tr>
<tr>
<td>DOM-1 acetate</td>
<td>([M-HOAc]^+), ([M-2HOAc]^+), ([M-3HOAc]^+), ([M-H]^+)</td>
<td>12 ± 0.6</td>
</tr>
</tbody>
</table>

*A-3-one has been assigned a relative response of 1.0; n = 3-4.*

References

(3) Hsu, C. S.; Dechart, G. J.; Rose, K. D. 38th ASMS Conference, June 1990, p. 595.
PRESSURE DEPENDENCE AND REGIOSELECTIVITY IN THE REACTIONS OF O\(^{-}\) WITH AROMATIC COMPOUNDS

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The gas phase chemistry of the oxide radical anion, O\(^{-}\), has been found to be useful in several important areas of chemistry. For instance, O\(^{-}\) has been shown to react rather selectively with halogenated pesticides and other environmental pollutants and has therefore been used as a negative ion chemical ionization reagent species. It has also been employed in the area of gas phase ion chemistry as a reagent to form not readily accessible gas phase organic anions. Finally, O\(^{-}\) chemistry has also been shown to play an important role in electron capture devices. In studies performed by other investigators, the principal ionic products in the reactions of toluene and benzene with O\(^{-}\) were [M-2H]\(^{+}\) and [M-H + O]\(^{+}\). In the case of toluene, oxygen incorporation has been assumed to be on the aromatic ring. We report results of experiments including reactions performed at different pressures, CID experiments, and isotope labelling which provide interesting information regarding the nature of O\(^{-}\) reactions with simple aromatic compounds.

The high pressure studies were performed on a Sciex TAGA 6000 triple quadrupole mass spectrometer equipped with an atmospheric pressure ionization source. The O\(^{-}\) reagent species were generated via dissociative electron capture by the N\(_{2}\)O carrier gas. The carrier gas was a mixture of 1% N\(_{2}\)O in N\(_{2}\). The laboratory collision energy was 80V and the collision gas was Argon. The low pressure experiments were performed on a VG-Quattro triple quadrupole mass spectrometer using the negative chemical ionization mode. The source housing pressure was 1.5x10\(^{-4}\) Torr while the pressure in the ion volume was presumably on the order of several Torr. The O\(^{-}\) reagent ions were formed efficiently by the use of N\(_{2}\)O alone as a negative chemical ionization gas. The laboratory collision energy was 110V and the collision gas was Argon.

Under atmospheric pressure ionization (API) conditions, the observed products have been previously reported to be [OH]\(^{+}\), at m/z 17 and [M-H + O]\(^{+}\) at m/z 107. The formation of a mixture of isomeric C\(_{6}\)H\(_{5}\)O\(^{-}\) ions was demonstrated by comparison of the CID spectra of such ions from toluene and four reference compounds. The results suggested the formation of a mixture of benzyloxide and ortho, meta, and para-methylphenoxides. The isomeric composition of this mixture was estimated using the concept of linear superposibility of CID spectra. The four most intense ions in the CID spectrum of m/z 107 from toluene were used for the analysis. The intensity of each fragment ion relative to the most intense fragment is tabulated in Table 1. By making a few assumptions an approximate ratio of o-methylphenoxide to the combined contribution of m-methylphenoxide and p-methylphenoxide can be determined. In addition, an approximate ratio of benzyloxide to o-methylphenoxide can also be determined, the primary assumption is that the m/z 64 fragment ion is contributed solely by the o-methylphenoxide isomer. The results are shown in Figure 1.

When the reactions of benzene and toluene with O\(^{-}\) were studied under API and negative chemical ionization (NCI), the effect of pressure on the product distribution was found to be pronounced. Under API conditions, evidence for proton transfer and dihydrogen transfer ([M-H]\(^{+}\) and [M-2H]\(^{+}\), respectively) in toluene or benzene was not observed. The only products observed were [OH]\(^{+}\) and [M-H + O]\(^{+}\) ions. A possible reason for the absence of [M-2H]\(^{+}\) in the API spectra may be that some species is present which quenches the highly reactive C\(_{6}\)H\(_{5}\)H\(^{+}\) ion forming a nondetectable neutral product. An alternate possibility is that the equilibrium conditions under API, as a result of the high collision rate, should favor the formation of the thermodynamically more stable product. The second possibility may also apply to the absence of [M-H]\(^{+}\). Under NCI conditions, where the collision rate is lower, benzene exhibits dihydrogen transfer while toluene exhibits both dihydrogen transfer and proton transfer. The API and the NCI spectra of toluene are shown below in Figures 2 and 3.

Of particular interest was the effect of perdeuteration of the methyl group in toluene on the reaction with O\(^{-}\). Under API an apparent change in the reactivity of toluene with O\(^{-}\) upon perdeuteration of the methyl group was observed. In this case, no ions characteristic of the behavior of toluene in the presence of O\(^{-}\) were formed. This suggests that the formation of [M-H + O]\(^{+}\) occurs via an initial hydrogen abstraction from the methyl group. These results, coupled with the predominant formation of the benzyloxide isomer of C\(_{6}\)H\(_{5}\)O\(^{-}\), also suggest that the formation of the methylphenoxide isomers of C\(_{6}\)H\(_{2}\)O\(^{-}\) is a result of rearrangement of the initially formed benzyloxide. An alternative possibility may be the rearrangement of the initially formed C\(_{6}\)H\(_{6}\) neutral species to a 7-membered ring which upon recombination with O\(^{-}\) may collapse to methylphenoxide.

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Under NCI conditions, the lower collision frequency and shorter residence time allow observation of reaction products analogous to [M-H]⁻, [M-2H]⁻, and [M-H + O]⁻. The spectrum of d₃-methylbenzene under NCI conditions is shown in Figure 4. Ions at m/z 91, 92, and 93, presumably corresponding to [M-2D]⁻, [M-HD]⁻, and [M-2H]⁻ or [M-D]⁻, respectively were observed. This mixture of products indicates hydrogen/deuterium scrambling and is in agreement with the 7-membered ring argument presented above. In addition, the formation of an oxygen substitution product appears to be suppressed, with a small amount of [M-D + O]⁻ being observed. This is also suggests that oxygen incorporation occurs primarily via hydrogen abstraction from the methyl group. Finally, the CID spectra of the m/z 107 and m/z 109 ions formed under NCI indicate the formation of a mixture of benzyloxy and methylphenoxides and the initial removal of an alkyl hydrogen in the pathway of oxygen incorporation. This is demonstrated by the CID of m/z 109 [M-D + O]⁻ under NCI conditions, shown in Figure 5, by the clean loss of 17 amu (-CD₂H) yielding m/z 92. The fragment ions at m/z 77, 78, and 79 in the same spectrum indicate losses of CD₂O, CDHO, and CH₂O, respectively. This suggests hydrogen/deuterium scrambling in the formation of the benzyloxide anion.

![Figure 1](image1)

![Figure 2](image2)

![Figure 3](image3)

![Figure 4](image4)

![Figure 5](image5)
GAS PHASE REACTIONS OF DITHIOLATE ANIONS WITH SELECTED LEWIS ACIDS

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Reactions of several dithiolate anions with BF₃ and SiF₄ have been carried out in the gas phase using the flowing afterglow technique. Specifically, the reactions of 1,2-ethanedithiol (HSEtS⁻), 1,3-propanedithiol (HSPrS⁻), 1,4-butanedithiol (HSBuS⁻), and 1,2-benzenedithiol (HSPhS⁻) have been examined. These species are of interest because they can react with the Lewis acid to form a monodentate addition product in a three-body step, and also present the Lewis acid center with a second functional group which can potentially react to form a bidentate product in a bimolecular step. In previous work several of the dioxo- analogs of these ions have been observed to form both monodentate and bidentate species in competing product channels. The observed mechanistic behavior of the dioxo- systems indicates that this class of reactions occurs on a double-well potential energy surface. The present work has been carried out in order to further examine factors influencing rates and product formation in these processes.

A summary of the products formed in these reactions is given in Table I. Upon examination of these results it is apparent that, in every reaction which proceeded, monodentate products were observed but no bidentate products are formed. Optimum geometries and single point energies for the monodentate and bidentate species for BF₃ + HSEtS⁻ have been obtained from ab initio calculations. These results indicate that, while formation of the monodentate product is energetically favored, bidentate product formation is near thermoneutral. This is likely due to the requirement that the large sulfur atoms be in close proximity to one another in the bidentate product. The only system which participates in bimolecular product formation is SiF₄ + HSEtS⁻ which forms SiF₃⁻ in addition to the monodentate product.

Table I

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Primary Addition</th>
<th>Secondary Addition</th>
<th>Tertiary Addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF₃ + HSEtS⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
</tr>
<tr>
<td>SiF₄ + HSEtS⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
</tr>
<tr>
<td>BF₃ + HSPrS⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
</tr>
<tr>
<td>SiF₄ + HSPrS⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
</tr>
<tr>
<td>BF₃ + HSBuS⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
</tr>
<tr>
<td>SiF₄ + HSbuS⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
</tr>
<tr>
<td>BF₃ + HSPhS⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
</tr>
<tr>
<td>SiF₄ + HSphS⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
<td>BF₄⁻</td>
</tr>
</tbody>
</table>

Figure 1
These two products are formed in parallel channels in a manner similar to that of the monodentate/bidentate formation in the dioxo- systems. The branching ratio between the bimolecular and three-body channels is influenced by the pressure. Thus, we conclude that in this system the double-well surface is also applicable. The general mechanism describing all the reactions studied here is given in Figure 1, where the bimolecular product channel is available only to the previously discussed reaction.

The lack of sulfide formation in the systems in which BF₃ is the Lewis acid is likely due to unfavorable energetics. In terms of bonds broken vs. bonds formed, sulfide formation when BF₃ is the Lewis acid is endothermic by 26 kcal-mol⁻¹, whereas the analogous process when SiF₄ is used is exothermic by 21 kcal-mol⁻¹. The fact that sulfide formation only occurs with HSeEtS⁻ can be explained if a mechanism involving fluoride attack on the α-carbon of the ligand is invoked. As the ligand chain length increases the α-carbon becomes less susceptible to nucleophilic attack by F⁻.

With the exception of the reaction of HSPhS⁻ with BF₃, the rates of these processes exhibit no significant pressure dependence and proceed at approximately 60% of their collision rates. The pressure dependent behavior of the observed rate constant for BF₃ + HSPhS⁻ is illustrated in Figure 2. The rate constants for this process range from 24% - 40% of the collision rate. Here also, we feel that this effect is due to the close proximity of the two sulfur atoms in this rigid ligand. The B-S interaction is weaker than in those systems where the second -SH group is free to move. Thus the dissociation back to reactants of the excited complex shown in Figure 1 occurs more frequently, resulting in a slower overall rate. This effect would also explain the lack of reaction of this anion with SiF₄ in which the Lewis acid center is even more crowded.

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Gas Phase Electron Transfer Reactions Between Selected Molecular Anions and Halogenated Methanes

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Ion-molecule reactions between eight halogenated methanes (RX), including CCl₄, CFCI₃, CF₂Cl₂, CHCl₃, CH₃I, CClBr₂, CF₃Br, and CH₂Br₂, with selected molecular anions (A'), including azulene (Az~), nitrobenzene (NB~) and several substituted nitrobenzenes using a pulsed high pressure mass spectrometer are reported. The electron affinities of the donor molecules (A) range from 16 kcal/mol for azulene to 46 kcal/mol for para-dinitrobenzene. The reaction rates show a steady decrease with increasing electron affinity of the donor molecule. The ionic reaction products were halide ions (X⁻) and clustered halide ions (A.X⁻). No molecular anions of the type RX⁻ were observed for any of the reaction systems.

In order to explain the products and rates of the reactions studied here (see Table I), a hybrid dissociative electron transfer mechanism is proposed. The overall process consists of two distinct reactions. The first reaction is rate determining and results in the transformation shown in Reaction 1:

\[ A' + RX \rightarrow A.X^- + R \]  \hspace{1cm} (1)

where the A.X⁻ cluster ion and radical (R) emerge from the reaction complex. Because the ionic product is a cluster ion, the proposed mechanism is called the CADET (cluster assisted dissociative electron transfer) mechanism. The second reaction involves the declustering-clustering equilibrium reaction:

\[ A.X^- \rightarrow A + X' \]  \hspace{1cm} (2)

The A.X⁻ cluster ion after emerging from the reaction complex is rapidly thermalized through collisions with the buffer gas. The relative intensities of X' and A.X⁻ observed in the mass spectrum reflect the equilibrium position of Reaction 2 which is governed by the gas temperature and partial pressure of NB.

The proposed reaction coordinate for the CADET mechanism is illustrated in Figure 1. Special stability for two intermediate species is envisioned, (A'.RX)' and (A.X'.R)'. The transition state which exists at the height of the energy barrier is thought to resemble a structure in which the C-X bond is being ruptured as an electron is being transferred from A' to the halide atom.

Many of the important features within the experimental results are readily explained in terms of the CADET mechanism.

1. The product ions observed were free halides (X') and their respective adducts (A.X⁻) which is consistent with the products anticipated by the decomposition of the (A.X'.R)' intermediate to A.X⁻ + R.

2. The decrease in the second order rate constants with increasing electron affinity of A is thermodynamically consistent with the proposition that Reaction 1 is the rate determining step. Figure 2 illustrates this point where the second order rate constants for the reactions of CCl₄, CClBr and CF₃Br₂ with various electron donors have been plotted against the enthalpy change of Reaction 1. The rate constants are found to decrease precipitously as \(-\Delta H^\circ\) approaches zero and are not detectable for any reactions where \(-\Delta H^\circ\) is less than zero.

3. The decrease in reactivity of the chlorofluorocarbons in the order CCl₄ > CFCI₃ > CF₂Cl₂ is only partially explainable based on the \(-\Delta H^\circ\)'s for Reaction 1. The stability of the transition state, and therefore the height of the internal energy barrier, is expected to be dependent on the polarizability of the radical leaving group. The polarizabilities of the radical leaving groups are predicted to decrease in the order CCl₄ > CFCI₃ > CF₂Cl₂. This decrease in polarizability leads to progressively less favorable transition states within this series, and thus lower reaction rates.

4. The low reactivity of CH₂Br₂ compared to CF₃Br₂ is thought to reflect the effect of dipole moment, as the differences in reaction exothermicity are too small, and the polarizabilities of the radical leaving group too similar to explain this observation. The anion-dipole interaction orients the CH₂Br₂ molecule within the transition state so that the Br atoms point away from A'. Electron transfer from A' is then more difficult than in the corresponding CF₃Br₂ ion complex where the Br atoms point towards A'.

5. The total lack of reactivity of CH₃I can be explained by a combination of both the low polarizability of the CH₃ radical and an unfavorable dipole-moment induced orientation of the CH₃I molecule within the transition state.
### TABLE I

Major Products and Rate Constants for Reactions Between Selected Molecular Anions (A') and Halogenated Methanes (RX). Rate constants shown in ( ) x $10^{-9}$ ml/s

<table>
<thead>
<tr>
<th></th>
<th>CCl₄</th>
<th>CFCl₃</th>
<th>CF₂Cl₂</th>
<th>CHCl₃</th>
<th>CH₃</th>
<th>CCl₃Br</th>
<th>CF₂Br₂</th>
<th>CH₂Br₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aثار</td>
<td>Cl⁻</td>
<td>Cl⁻</td>
<td>n.d.</td>
<td>Cl⁻</td>
<td>n.d.</td>
<td>Br⁻  (14.0)</td>
<td>Br⁻ (8.2)</td>
<td>Br⁻ (0.07)</td>
</tr>
<tr>
<td>o-FNB⁻</td>
<td>Cl⁻  (0.83)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Br⁻  (6.5)</td>
<td>Br⁻ (3.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m-FNB⁻</td>
<td>Cl⁻  (0.05)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Br⁻  (5.9)</td>
<td>Br⁻ (0.47)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>o-NO₂NB⁻</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Br⁻  (0.47)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-NO₂NB⁻</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Proposed reaction coordinate for the CADET mechanism. The relative energies indicated correspond to the $A = NB, RX = CCl₄$ reaction system. Well depths assigned for the two intermediates have been estimated.

Figure 2. Observed second order rate constants at 125°C for the reactions of CCl₄ (o), CCl₃Br (x) and CF₂Br₂ (D) with molecular anions as a function of the $\Delta H$ of Reaction 1.
Isotope Exchange and Association Reactions of Hydroxide and Alkoxide Clusters

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University of Colorado at Boulder

Hydrogen/deuterium (H/D) exchange reactions of methoxide-water clusters with a variety of labeled water reagents were studied using the Tandem Flowing Afterglow Selected Ion Flow Tube (FA-SIFT). For these reactions, multiple proton transfers may occur within the complex. Table 1 summarizes the results of the exchange reactions. For those systems where more than one possible product was formed, the branching ratio for the endothermic channels was statistical while the exothermic channels were nonstatistical. The statistical results implicate formation of a relatively long lived complex which gives rise to complete scrambling.

TABLE 1. Rate Coefficients, Reaction Efficiencies, Branching Ratios, and Reaction Enthalpies

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate Coefficient</th>
<th>Reaction Efficiency</th>
<th>Branching Ratio</th>
<th>Reaction Enthalpy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \times 10^{-5} ) cm³ particle⁻¹ s⁻¹</td>
<td></td>
<td></td>
<td>kcal mol⁻¹</td>
</tr>
<tr>
<td>1 a. ( \text{CH}_3\text{O}^- + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{O}^- + \text{H}_2\text{O} )</td>
<td>1.06 (±0.10)</td>
<td>39%</td>
<td>80%</td>
<td>80%</td>
</tr>
<tr>
<td>2 a. ( \text{CH}_3\text{O}^- + \text{D}_2\text{O} \rightarrow \text{CH}_3\text{O}^- + \text{D}_2\text{O} )</td>
<td>1.37 (±0.16)</td>
<td>52%</td>
<td>35%</td>
<td>36%</td>
</tr>
<tr>
<td>3 a. ( \text{CH}_3\text{O}^- + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{O}^- + \text{H}_2\text{O} )</td>
<td>1.29 (±0.05)</td>
<td>49%</td>
<td>28%</td>
<td>33%</td>
</tr>
<tr>
<td>4 a. ( \text{CH}_3\text{O}^- + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{O}^- + \text{H}_2\text{O} )</td>
<td>1.45 (±0.07)</td>
<td>53%</td>
<td>43%</td>
<td>33%</td>
</tr>
<tr>
<td>5 a. ( \text{CH}_3\text{O}^- + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{O}^- + \text{H}_2\text{O} )</td>
<td>1.70 (±0.19)</td>
<td>60%</td>
<td>60%</td>
<td>80%</td>
</tr>
<tr>
<td>6 a. ( \text{CH}_3\text{O}^- + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{O}^- + \text{H}_2\text{O} )</td>
<td>1.57 (±0.14)</td>
<td>57%</td>
<td>40%</td>
<td>20%</td>
</tr>
</tbody>
</table>

a Number in parenthesis is one standard deviation of experimental rate coefficients.
b \( k_{\text{obsd}} / k_{\text{VAR}} \) and \( k_{\text{VAR}} \) calculated according to Chesnavich, W. J., Su, T.; Bowers, M. T. J. Chem. Phys. 1980, 72, 2641.
c BR_{\text{stat}} are the statistical branching ratios assuming complete scrambling within the complex.
d BR_{\text{expt}} are the experimental branching ratios.
e The absolute errors in the branching ratios are estimated to be less than 10% of the percentage reported.

The AH_{\text{rxn}} signs are qualitatively assigned. It is known that substituting a deuterium for a hydrogen in anions is energetically unfavorable. (Grabowski, J. J., DePuy, C. H.; Van Doren, J. M.; Bierbaum, V. M. J. Am. Chem. Soc. 1985, 107, 7384).

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In addition, the rate coefficients for the three body association reactions which form hydroxide-water, methoxide-water, methoxide-methanol and ethoxide-ethanol and their various deuterated analogs were measured as a function of helium pressure. Figure 1 gives the experimental data for the association reactions of hydroxide and alkoxides. The apparent bimolecular rate coefficient \( k_{app} \) of the following fourteen reactions is plotted against the helium pressure.

\[
\begin{align*}
H_0^- + H_2O &\rightarrow HO^-\cdot H_2O \\
D_0^- + D_2O &\rightarrow DO^-\cdot D_2O \\
CH_3O^- + H_2O &\rightarrow CH_3O^-\cdot H_2O \\
CH_3O^- + D_2O &\rightarrow CH_3O^-\cdot D_2O \\
CD_3O^- + H_2O &\rightarrow CD_3O^-\cdot H_2O \\
CD_3O^- + D_2O &\rightarrow CD_3O^-\cdot D_2O \\
CH_3O^- + CH_3OH &\rightarrow CH_3O^-\cdot HCH_3 \\
CH_3O^- + CH_3OD &\rightarrow CH_3O^-\cdot DOCH_3 \\
CD_3O^- + CD_3OH &\rightarrow CD_3O^-\cdot HOCD_3 \\
CD_3O^- + CD_3OD &\rightarrow CD_3O^-\cdot DOCD_3 \\
CH_2CH_2O^- + CH_2CH_2OH &\rightarrow CH_2CH_2O^-\cdot HOCCH_2CH_3 \\
CD_2CH_2O^- + CD_2CH_2OH &\rightarrow CD_2CH_2O^-\cdot HOCCH_2CD_3 \\
CH_2CD_2O^- + CH_2CD_2OH &\rightarrow CH_2CD_2O^-\cdot HOCD_2CH_3 \\
CD_2CD_2O^- + CD_2CD_2OD &\rightarrow CD_2CD_2O^-\cdot DOCD_2CD_3
\end{align*}
\]

The lifetimes of the clusters were determined. The results indicate that deuterating the alkyl groups significantly lengthens the lifetime of the clusters, while deuterating the bridging position has very little effect on the lifetime of the clusters. This implies that the methyl groups are nearly free rotors in the complex, thus providing large numbers of states to store the energy; on the other hand, the newly formed OH--O bond produces only vibrational modes with much lower density of states.
DEHYDROGENATION OF ETHANE BY CpM⁺ (M=Fe, Co AND Ni):
AN FTICR STUDY

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The reactivity of transition metal ions with alkanes is influenced by the ligands on the
metal. Fe⁺, for example, does not react with ethane, but FeL⁺ (L=CH₂, CH₃, C₅H₆) does react
with ethane to give a series of products [1], [2]. In these cases the reactivity of Fe⁺ is enhanced
by participation of ligands in the reactions. CpCo⁺ has been found to dehydrogenate ethane[3],
but reactions of CpFe⁺ and CpNi⁺ with ethane have not been reported. Using FTICR
techniques we have found reactions of CpM⁺ (M=Fe, Co and Ni) with ethane as follows:

\[
\begin{align*}
\text{CpFe}^+ & \quad + \quad C_2H_6 \quad \rightarrow \quad \text{FeC}_3H_5^+ \quad + \quad C_4H_6 \quad \quad (1) \\
\text{CpCo}^+ & \quad + \quad C_2H_6 \quad \rightarrow \quad \text{CpCoC}_2H_4^+ \quad + \quad H_2 \quad \quad (2) \\
\text{CpNi}^+ & \quad + \quad C_2H_6 \quad \rightarrow \quad \text{CpNiC}_2H_4^+ \quad + \quad H_2 \quad \quad (3)
\end{align*}
\]

The ratio of \( k_{\text{Fe}} \): \( k_{\text{Co}} \): \( k_{\text{Ni}} \) is 0.01: 1.0: 0.4. In addition the following subsequent reactions at
high pressure in the Fe system were observed.

\[
\begin{align*}
\text{FeC}_3H_5^+ & \quad + \quad C_2H_6 \quad \rightarrow \quad \text{FeC}_5H_9^+ \quad + \quad H_2 \quad \quad (4) \\
\text{CpFe}^+ & \quad + \quad C_2H_6 \quad \rightarrow \quad \text{CpFeC}_2H_6^+ \quad \quad (5)
\end{align*}
\]

These observations raise the question of why CpFe⁺ reacts in such a completely
different manner from CpCo⁺ and CpNi⁺. We offer the following interpretation in terms of
Frontier Orbital Theory.

First consider an MO diagram for CpFe⁺ as shown in Fig. 1. From this diagram we see
that all bonding MOs are occupied and the LUMOs are ligand based \( \epsilon_1^\prime \) orbitals. The \( \epsilon_1^\prime \)
LUMOs are depicted in Fig. 2. Indicated are the coefficients of the \( 2p_z \) orbitals on the carbons
and the \( d_{xz} \) (\( d_{yz} \)) orbital on Fe. The 12 valence electrons occupy bonding orbitals giving a
strong metal ligand interaction and a LUMO with C-C antibonding character leading to C-C
bond cleavage on reaction. This is why for CpFe⁺ the Cp ring is involved in the reaction.

In CpCo⁺ and CpNi⁺ occupation of antibonding orbitals will weaken the interaction
between Cp and M⁺. The MO diagram for CpCo⁺ is shown in Fig. 3 (CpNi⁺ would be
similar). This diagram tells us that \( d \) orbitals are not strongly perturbed by the ligand and thus
occupation in a high spin sense is expected. The resulting LUMO becomes the \( a'' \)
orbital which is strongly metal 4s in character as depicted in Fig. 4. Indicated are coefficients of the
\( 2p_z \) orbitals on the carbons and 4s orbital on the metal. With 13 and 14 valence electrons the
CpCo⁺ and CpNi⁺ have occupied antibonding orbitals resulting in a weak metal-ligand
interaction. The LUMO has strong metal 4s character and the metal is more like a free metal
with \( d \) orbitals occupied in a high spin sense. The metal 4s LUMO leads to dehydrogenation
reactions.

Fig. 1

Fig. 2

Fig. 3

Fig. 4
**C₃H₄N₂O₃⁻ Anions Produced by Reaction of 2-Chloro-5-Nitropyridine with the Hydroxide Ion**

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**Introduction**

In solution and also in the gas phase, the electron deficient nature of the pyridine ring makes the electrophilic aromatic substitution reactions that are typical of aromatic systems very difficult, but instead facilitates nucleophilic aromatic substitution reactions. Nucleophilic aromatic substitution occurs in solution by three separate mechanisms (1), the addition elimination mechanism, $S_N(\text{AE})$, the benzene mechanism, and the aromatic nucleophilic substitution by ring opening and ring closing mechanism $S_N(\text{ANRORC})$. The $S_N(\text{ANRORC})$ mechanism has received much less attention than the other two mechanisms and it has not been much investigated by mass spectrometry.

**Solution Reaction**

The particular reaction of interest in this study is the reaction of 2-chloro-5-nitropyridine with OH⁻ (2,3). In a solution of dimethylsulfoxide and water this reaction forms a stable, isolatable, ring-opened $C_3H_3N_2O_3$⁻ anion whose structure has previously been determined by NMR and is shown in Figure 1. Addition of excess OH⁻ closes the ring again and leads to the expected nucleophilic addition product, the 2-oxy-5-nitropyridine anion. Both products are salts and are isomers of each other. Negative ion fast atom bombardment using glycerol as the matrix successfully desorbs the molecular anions of both products. Neither low nor high energy negative ion CID of these ions differentiates the two isomers. However, negative ion fast atom bombardment combined with high energy charge inversion tandem mass spectrometry (4) in a Kratos MS-50 three sector EBE instrument is an excellent means of differentiating the two ions from the $S_N(\text{ANRORC})$ solution-phase reaction. Figure 1 shows the charge inversion tandem mass spectra of the two products. Probable formulas for the positive fragment ions of the negative ion precursors are shown above the appropriate peaks in Figure 1.

**Gas-Phase Reaction**

In a negative ion chemical ionization source, OH⁻ ions derived from water reacted with 2-chloro-5-nitropyridine in the gas phase to produce an ion with a charge inversion tandem mass spectrum (see Figure 2) that is identical to that of the 2-oxy-5-nitropyridine anion. Apparently this analogous gas-phase reaction follows an $S_N(\text{AE})$ mechanism since the ring-opened ion is not detected. Scheme 1 shows simplified mechanisms for both of the solution and gas-phase reactions of 2-chloro-5-nitropyridine with OH⁻.

**Acknowledgement**

I would like to thank M.L. Gross from the University of Nebraska-Lincoln, and J.T. Watson from Michigan State University for allowing me to use their tandem mass spectrometers for this research.

**References**

Figure 1A: Charge Inversion FAB/MS/MS Spectrum of

Figure 1B: Charge Inversion FAB/MS/MS Spectrum of

Scheme 1: Mechanisms for the Solution and Gas-Phase Reactions

1. \( \text{H}_2\text{N}-\text{Cl} \xrightarrow{\text{OH}^-} \text{H}_2\text{O} + \text{H}_2\text{N}-\text{Cl} \) (in Solution)

2. \( \text{H}_2\text{N}-\text{Cl} \xrightarrow{-\text{HCl}} \text{H}_2\text{O} + \text{H}_2\text{N}-\text{Cl} \)

3. \( \text{H}_2\text{N}-\text{Cl} \xrightarrow{-\text{HCl}} \text{H}_2\text{O} + \text{H}_2\text{N}-\text{Cl} \) (in Gas Phase)
INTRODUCTION
Reaction of C_3H_3^+ with acetylene has received considerable attention in recent years. The high concentration of C_3H_3^+ ions in sooting flames has suggested that this cation plays an important role in the ionic root to the formation of the soot. Reaction of linear-C_3H_3^+ with acetylene and C_2D_2 in FT/ICR has shown that linear-C_3H_3^+ isomerizes to the cyclic form. Isomerization was shown to take place via a long-lived C_5H_5^+ collision complex by hydrogen exchange reactions between linear-C_3H_3^+ and C_2H_2. Better understanding of the isomerization of linear C_3H_3^+ was achieved using C_2D_2 instead of C_2H_2. C_3H_3^+, C_3H_2D^+, C_3HD_2^+ and C_3D_3^+ ions were produced by isotope exchange reactions. To further investigate this isomerization mechanism, i.e., to examine if this isomerization involves skeletal exchange, reaction of linear C_3H_3^+ with ^13C_2-acetylene was studied in a quadrupole ion trap mass spectrometer.

EXPERIMENTAL
A Finnigan ion trap mass spectrometer was used for this study. C_3H_3^+ was produced by charge-transfer reaction of propargyl bromide with Xe^+, formed by electron impact ionization. Ejection capabilities of the ITMS were used for this charge transfer reaction and for the isolation of the C_3H_3^+. Neutral gas pressures were measured with an ionization gauge. P_{C_3H_3Br}=2\times10^{-7} \text{Torr}; P^{13C_2H_2}=4\times10^{-7}; PXe=1.5\times10^{-6}. Pressure of helium was adjusted to a total ionization gauge reading pressure of 8\times10^{-5}, i.e., 1 \text{ mTorr}.

RESULTS AND DISCUSSION
Reactivity of C_3H_3^+. Reactivity of the C_3H_3^+ was monitored by observing reaction with precursor neutrals. Percentage of reactive (linear) C_3H_3^+ formed by chemical ionization charge transfer from Xe^+ was 75% (Figure 1), consistent with the FT/ICR study.

Isomerization of Linear C_3H_3^+. After ejection of all ions except C_3H_3^+ following charge-transfer chemical ionization by Xe^+ of a mixture of ^13C_2H_2 and propargyl bromide, the ion/molecule reactions as a function of time were monitored. It was observed that (see Figure 2): 1) Reactions with ^13C_2H_2 led to the isomerization of linear-C_3H_3^+ to the non-reactive cyclic-C_3H_3^+. 1600
2) Isomerization occurs through a C₅H₅⁺ reaction complex via skeletal exchange producing

\[ ^{13}\text{C}_2\text{H}_3^+, \; ^{13}\text{C}_2\text{CH}_3^+, \text{ and } ^{13}\text{C}_3\text{H}_3^+. \]

Collisional Stabilization of the C₅H₅⁺ Collision Complex

In the pressure regime of the FTMS, no C₅H₅⁺ ion was observed when C₃H₃⁺ reacted with acetylene, indicating that most of the C₅H₅⁺ collision complex dissociated to give the cyclic C₃H₃⁺, rather than linear isomer. Since quadrupole ion traps operate at higher pressure, effect of pressure on the C₅H₅⁺ formation was studied using helium buffer gas for third-body stabilization of the C₅H₅⁺ collision complex. Intensity of the C₅H₅⁺ increased, directly, when the system pressure was increased from \(2 \times 10^{-6}\) to \(5 \times 10^{-4}\) torr (uncorrected ion gauge reading), supporting the collision complex formation mechanism (Figure 3).

CONCLUSION

The results show that the isomerization of the linear-C₃H₃⁺ to the cyclic form occurs through a C₅H₅⁺ reaction complex via hydrogen and/or skeletal exchange. Third body stabilization of the C₅H₅⁺ occurs at pressures above 1 mtorr, supporting the collision complex formation mechanism.

REFERENCES

Formation of Substituted Benzene Radical Cations via Ion/Molecule Reactions with Iodobenzene

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Ion/molecule reactions commonly occur in mass spectrometers. While in many cases such reactions are undesirable they can be useful for structure determination or for comparison of gas-phase chemistry with solution chemistry. In general, when an even electron ion reacts with a neutral molecule, the product ion formed is also an even electron ion due to the normally unfavorable energetics involved in electron unpairing. A notable exception to this is a system we have been studying, the reactions of iodobenzene with a variety of different even-electron ions.

As shown below, a variety of even-electron hydrocarbon ions react with iodobenzene to give alkyl benzene radical cations. The general reaction is

\[ R^* + I^- \rightarrow \phi + \phi + R^* + I^- \]

Thus, an ion is observed at \((R+77)\). Reactions are observed in cases in which the reactant ion is less than 15 kcal/mol or so more stable than the resulting alkylbenzene radical cation. The driving force for the reactions in which the odd-electron product ion is less stable than the even-electron reactant ion is the fairly unique situation of the radical product \((I^*)\) being about 15 kcal/mol more stable than the neutral reactant (iodobenzene).

We first observed the reaction of iodobenzene giving substituted benzene radical cations in the methane CI mass spectrometry of iodobenzene. With the ion trap, selected reagent ion CI demonstrated that \(C_6H_4I^+\) gives an ion at m/z 106, \(C_6H_5I^+\), which is ethylbenzene. While the fact that this reaction occurs is interesting, the product can reasonably only be ethylbenzene. However, the next higher ion in the series, the propyl cation, has two possible structures \(n-\) or \(i-\). Thus, m/z 43 is allowed to react for 100ms is shown in Figure 1. The major product ion is m/z 57 due to hydride abstraction from isobutane. The other two ions in the spectrum result from reactions of 43 with \(\phi\). The ion at m/z 205 is formed by the commonly observed proton transfer reaction while the species at m/z 120 corresponds to the substitution of \(C_6H_4I^+\) for \(I^-\) in \(\phi\). The thermochemistry of the system is that the proton transfer is exothermic by about 2 kcal/mol, \(\Delta H_{298}^0\) substitution is exothermic by about 5 kcal/mol and \(n-\) \(C_6H_4I^+\) substitution is exothermic by about 25 kcal/mol.

Figure 2 shows the MS\(^3\) experiment in which the MS/MS spectrum of the reaction product at m/z 120 is obtained. From comparison of this spectrum with MS/MS spectra of the two isomeric propylbenzenes, it can be determined that the expected isopropylbenzene is the major product (giving the product ion at m/z 105) but that there is some \(n-\) propylbenzene also formed (giving product ions at m/z 91 and 78). Continuing up in the series, \(C_6H_5I^+\) would not be expected to react if it is \(n\)-butyl but the other three isomers, \(s-\), \(i-\), and \(n-\) butyl should react. When m/z 57 is formed from 1 or 2-chlorobutane approximately 0.06% of the ions are reactive, giving m/z 134. The product ion spectrum of m/z 134 has only one ion, m/z 105, which is characteristic of \(s-\) butylbenzene.

The vinyl cation is an extremely reactive species which rarely is observed to form stable substitution products because of the high exothermicity of its reactions. However, when it reacts with \(\phi\) the substitution product is observed. An MS\(^3\) experiment was performed in which the MS/MS product ion spectrum was obtained on the vinyl cation reaction product with the vinyl cation being formed by CID of \(C_6H_4I^+\). This spectrum indicated that styrene is formed, as expected. This reaction is about 50 kcal/mol exothermic, which suggests that significant energy may be partitioned into translational energy of the products.

An ion with a higher degree of unsaturation than the vinyl cation, \(C_6H_5^+\), which is a major fragment from phenyl cations, has also been studied. In addition to the product ion formed from the \(\phi\) substitution reaction, the molecular ion and protonated molecule of \(\phi-I\) are observed. The protonated species comes about as discussed above. The molecular ion is formed by charge exchange with \(^{13}C_C_6H_4I^+\) isobaric which is also present at m/z 51 when that m/z is selected from the mass spectrum. (Isobarically pure m/z 51 can be generated by MS/MS if so desired.) The MS/MS spectrum of the product ion formed by \(C_6H_5^+\) is shown in Figure 3. MS/MS experiments generating \(^{13}C\) containing \(C_6H_5^+\) suggest that the structure of m/z 128 is that of naphthalene. The MS/MS spectrum of the molecular ion of naphthalene was obtained and is identical to Fig. 3.


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The 39th ASMS Conference on Mass Spectrometry and Allied Topics
During the course of the MS/MS experiments on the m/z 128 reaction product it was observed that the product ion at m/z 127 produced an ion at m/z 204. While this could be the molecular ion of I⁻, it is also (R+77) for R=127. An MS³ experiment demonstrates that it is the later species which is formed.

The reaction of m/z 127 indicated that cyclic ions would also react in addition to the linear hydrocarbons. Figure 4 shows the reaction of the phenyl cation with I⁻. Not only is the substitution product observed but also the reaction intermediate, m/z 281. From a kinetics plot for the reaction m/z 77 the rate constant for this reaction in the ion trap was determined to be 2 x 10⁻⁹ cm²/s/molecule, indicating that this is a very efficient reaction. The MS/MS spectrum of the reaction intermediate shows the expected dissociation, loss of I⁻. The MS/MS spectrum of the reaction product indicates that it is biphenyl.

We have observed this reaction with other halobenzenes. Crude bracketing of unknown heats of formation can be performed by observing if this reaction occurs with I⁻ and the analogs, Br and Cl. The reaction will occur with I⁻ if the difference in heats of formation of the reactant and product ion is less than 15 kcal/mol. For the reaction to occur with Br the product ion must be at least as stable as the reactant, while for Cl the product ion must be at least 15 kcal/mol more stable than the reactant.
ORIGIN OF [M+C_nH_{2n-2}] ANIONS IN THE NCI MASS SPECTRA OF SUBSTITUTED PAHs WHEN USING METHANE AS THE BUFFER GAS

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Colin Moore,
Kratos Analytical Inc., 535 East Crescent Avenue, Ramsey, NJ. 07446.

Polycyclic aromatic hydrocarbons (PAHs) have long been recognized as hazardous environmental pollutants because of their carcinogenic effect on man. Attempts have been made to utilize many chromatographic and/or spectroscopic techniques for the analysis of these compounds. Adduct ions of [M+14]' and [M+28]' were reported in mass spectra of many PAHs when the negative chemical ionization (NCI) technique was used, but their formation has been attributed to two totally different reactions occurring in mass spectrometer (MS) source. The first reaction involves the substrate and hydrocarbon buffer gas in the ion source and results in the formation of [M+C_nH_{2n}]' adduct ions (ref. 1). The second reaction is a result of a wall-catalyzed oxidation reaction (ref. 2) giving a [M+14]' adduct. In the GS/MS analysis of PAHs under methane negative chemical ionization conditions, we observed significant abundances of [M+12]', [M+26]', [M+40]', ...[M+C_nH_{2n-2}]' ions in the NCI mass spectra of substituted fluorenes (Fig. 1). The results from a detailed study as to the origin of these adduct anions are reported here.

The experiments were performed using Hewlett-Packard 5890 gas chromatographs coupled via a capillary direct interface to either a quadrupole MS (Hewlett-Packard 5988A) or a magnetic sector MS (Kratos CONCEPT IS). The relative intensities of the adduct ions were similar for both types of mass spectrometer. The compounds of interest were fluorene, 1-methyl-fluorene, 11H-benzo[a]fluorene, and 11H-benzo[b]fluorene. The benzene solution of the compound was injected in splitless mode onto a DB-17 column (30m x 0.25um i.d.) which was temperature programmed as follows: 90°C for 1 min, ramped at 20 °C min^{-1} to 250 °C and held for 10 min. Helium was used as the carrier gas after being filtered through an OMI-1 indicating purifier. The ion source was at 150 °C for all experiments. The indicated methane or argon buffer gas pressure was 1 x 10^{-5} torr measured in the source housing, corresponding to an estimated source pressure of 0.1 torr. For the accurate mass measurements, the sample was introduced into MS via direct insertion probe and the mass spectrometer resolution was set at 6000 (10% valley definition). Peak matching against pfk reference ions was then used to determine the exact mass of the ion of interest.

The [M+C_nH_{2n-2}] anions were not observed when argon was used as the reagent gas, which indicated that the adducts were probably due to reaction of substituted fluorenes to the species from the methane gas. The results from accurate mass measurements of these adduct anions were given in Table 1. These data confirmed that the [M+12]' was [M+C]', [M+26]' was [M+C_2H_2]', and [M+40]' was [M+C_3H_4]'. It is interesting to note that the most abundant adduct ion is [M+C]', and then [M+C+CH_2]', [M+C+C_2H_4]', ...[M+C+C_nH_{2n-2}]' and that substituted fluorenes have one unsaturated site on their aromatic rings; while other PAHs without unsaturated sites only form [M+C_nH_{2n}]' ions. This observation suggested that substituted fluorenes may react with hydrocarbon species in the methane buffer gas at the unsaturated site and rearrange to a saturated PAH as shown in the following equation.
Figure 1. NCI mass spectra of fluorene, 1-methylfluorene, 11H-benzo[a]fluorene, and 11H-
benzo[b]fluorene introduced through the gas chromatograph at a source temperature of 150° C using
CH₄ as the reagent gas.

TABLE 1. PREDICTED AND OBSERVED MASSES FOR ELEMENTAL FORMULA OF
SUBSTITUTED FLUORENES

<table>
<thead>
<tr>
<th>Compound</th>
<th>C₁₄H₁₀ predicted</th>
<th>C₁₄H₁₀ observed</th>
<th>C₁₅H₁₂ predicted</th>
<th>C₁₅H₁₂ observed</th>
<th>C₁₆H₁₂ predicted</th>
<th>C₁₆H₁₂ observed</th>
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<tr>
<td>Fluorene</td>
<td>178.0783</td>
<td>178.0784</td>
<td>192.0939</td>
<td>192.0936</td>
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<td>-</td>
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<td>1-Methylfluorene</td>
<td>-</td>
<td>-</td>
<td>192.0939</td>
<td>192.0926</td>
<td>-</td>
<td>-</td>
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<tr>
<td>11H-Benzo[a]fluorene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>228.0939</td>
<td>228.0945</td>
</tr>
<tr>
<td>11H-Benzo[b]fluorene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>228.0939</td>
<td>228.0943</td>
</tr>
</tbody>
</table>

Further investigations of the adduct ions using model compounds and MS/MS techniques are
expected to provide proof of their structural identities and give more information about reaction
pathways of their formation.

REFERENCES
Carbon Cluster Ion Reactivity with Aromatic and Polyaromatic Hydrocarbons

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The reactivity of carbon cluster ions (C_n^+, 10<n<25) has been studied with various aromatic and polyaromatic hydrocarbons in a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer. Reactions proceed by one or more of three routes: charge transfer [1], adduct, and/or adduct minus a hydrogen atom ([adduct-H]) ion formation. Carbon cluster ions of this size are generally believed to be monocyclic rings which display limited reactivity due to the lack of exposed carbon atoms[2]. However, these cluster ions were discovered to react either by adduct or [adduct - H] ion formation dependent of whether the cluster contained an even- or odd-number of carbon atoms. This alternating reactivity suggests different structures and perhaps the presence of structural isomers.

Collisionally induced dissociation (CID) of the reaction products (adduct or [adduct-H] ions) produced limited fragmentation (loss of hydrogen atoms and molecules, C_2H_2(C_2)) for adducts formed with PAH's or styrene, and loss of HNC (HCN) and CH_3 from aniline and toluene adducts, respectively. No original reactants were created. At sufficient collision energies bare carbon cluster ions were formed (cf. Fig. 1, the CID of the [Cu.CioHg] ion). Similar results were obtained for the diadducts. These results imply the adducts are formed via multiple bonds with PAH type conjugation and are not loose associations. It was also possible to grow large adducts, associating to a maximum of four, that are rich in dehydrogentation. This suggests that carbon cluster ion/neutral reactions may contribute to the ion distributions observed in laser ablation of carbon containing materials.

Detailed investigation of the cluster ion reactivity with naphthalene reveals several interesting characteristics. All reactions (both products and reaction rates) were found to have a profound dependence on the kinetic energy of the cluster ion and on the pressure of a bath gas. Figure 2 shows both of these effects for the reaction of C_n^+ and naphthalene (all spectra obtained at the same naphthalene pressure and reaction time). The spectra shown start at high ion kinetic energies, with the center spectrum at ca. thermal ion kinetic energies. The last two spectra were acquired at background argon pressures of 1 x 10^-6 and 1 x 10^-5 torr, respectively. The rate constant for adduct formation decreased as the charge transfer rate increased as a function of increasing ion kinetic energy. Figure 3 shows computer modelled fits to the data for C_20^+ and naphthalene. The charge transfer and adduct rate constants were varied on the time scale of the experiment as a function of unreactive collisions.

The kinetics (in naphthalene reactions) indicate C_{11}^+, C_{19}^+ and C_{19}^+ have structural isomers, forming both adduct and [adduct-H] ions and exhibiting bi-exponential kinetics are observed for these ions. The lifetimes of the ion/molecule complex are estimated using a computer program that models the kinetics using radiative lifetimes ranging from 7 to 100 s^{-1} [3]. This produces lifetimes which are on the order of 0.1 - 3 ms, much longer-lived than
anticipated. These findings were verified by varying the argon bath gas pressure to collisionally stabilize the ion/molecule complex. In addition, experiments were performed to estimate the kinetic energy of the desorbed ions. Ions were first thermalized and then subjected to a single frequency excite pulse to increase their kinetic energy to a known value. Figure 4 shows the results for C_{15}^+. The enclosed area represents unthermalized results and was abnormally high, 4 - 6 eV. A 2 volt trapping potential was used, therefore considerable kinetic energy must be present as cyclotron motion.


Figure 1

Figure 2

Figure 3

Figure 4
GAS-PHASE MOLECULAR RECOGNITION:
ALKALI METAL ION TRANSFER REACTIONS OF CROWN ETHERS

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Background. The special ability of crown ethers to selectively form complexes with alkali metal cations in solution is well known. This selectivity is generally believed to be the result of a size match between the ligand cavity and an alkali metal cation; cations which match the cavity size bind more strongly than those which do not. In addition, the stabilities of crown-cation complexes are known to have strong solvent and counterion dependence. For example, the complex of 15-crown-5 with K+ is more stable in aqueous and methanolic solutions than the corresponding Na+ complex, although calculations indicate that the intrinsic M+-crown bond strength is greater for M = Na than for M = K. These species have also been the subject of recent theoretical work.

Motivation. The present study is motivated in part by the need to obtain data which are directly comparable to theoretical studies, i.e., gas phase data where solvents and counterions are not present. Crown-cation complexes have been observed previously using FAB and 252Cf-plasma desorption techniques. However, in all of these studies it is unclear whether complex formation occurred in solution prior to volatilization or in the gas phase after volatilization.

Size- and shape-specific chemistry is rarely observed in the gas phase. Crown ether-alkali cation experiments provide an opportunity to probe the relationship between cation radius, crown cavity radius, and reactivity, since the alkali metal cations are chemically quite similar except for their size.

Experimental. We have successfully generated gas-phase complexes of Na+, K+, Rb+, and Cs+ with 15-crown-5 and 18-Crown-6 using laser desorption. Solutions of crown ether and cation salts (generally chlorides or nitrates) in methanol were deposited on a stainless steel probe. Solvent was removed and the probe was inserted into a vacuum chamber and located immediately adjacent to the trapping cell of a Nicolet/Extrel FTMS-1000 ion cyclotron resonance mass spectrometer. Complex ions are trapped in the cell following irradiation of the probe tip with the focussed output of an excimer-pumped dye laser. Very small pulse energies are required (less than the 0.5 mJ/pulse threshold of our detector), provided the probe surface lies within a few mm of the focal point of a 150 mm fl lens. Subsequent ion-molecule chemistry is carried out by admitting neutral reagents through a leak valve and monitoring ion populations as a function of reaction time. Collision-induced dissociation (CID) and threshold CID experiments were performed on a Finnigan TSQ-70 using fast atom bombardment-generated complexes.

Results and Discussion. We believe the species we are generating using desorption methods are intact crown ethers complexed to metals. Low-energy CID of the same complexes generated using fast atom bombardment gives only the alkali metal cation as a major fragment. Likewise, laser desorption produces primarily ions of the expected complex mass with little fragmentation.

All of the 15-crown-5/alkali cation complexes studied are observed to transfer the cation to 18-Crown-6 when neutral crown is admitted into the vacuum system. This is in good agreement with experiments in solution and with theory, all of which indicate that these cations bind 18-Crown-6 more strongly than 15-Crown-5. Rate studies indicate these reactions are relatively
inefficient, as seems reasonable since proper cavity-to-cavity orientation is probably essential for a reactive collision. The reverse reactions, transfer of cation from 18-Crown-6 to 15-Crown-5, are not observed after reaction times of 30 seconds.

Surprisingly, in addition to the expected transfer reactions, formation of \((\text{crown})_2\text{M}^+\) is observed. Evidently, these species can radiatively cool rapidly enough to stabilize the collision complexes. Kinetic plots support this idea, showing that simple pseudo-first order kinetics are not obeyed for these reactions. Species of the same stoichiometry have been observed in condensed media and have “sandwich” structures, with the cation lying between two parallel macrocyclic rings.\(^1\)

The formation of these alkali cation bound “sandwich” dimers can be understood in terms of the relative sizes of the cations and crown ether cavities, if it is assumed that the cation must protrude significantly from the ring cavity in order for “sandwich” building to occur. All of the studied cations form sandwiches with 15-Crown-5, consistent with the idea that none of them can fit within the cavity. Likewise, only Cs\(^+\) forms a sandwich with 18-Crown-6. Na\(^+\) and K\(^+\) have radii less than that of the 18-Crown-6 cavity, and Rb\(^+\) is only slightly larger, so these cations lie mostly within the ring cavity. Cs\(^+\) is much too large to fit within the cavity and therefore is accessible for “sandwich” formation. The mixed \((15\text{-Crown-5})(18\text{-Crown-6})\text{M}^+\) complexes are also explained by this model. Na\(^+\) and K\(^+\) can fit within 18-Crown-6 and do not form a mixed sandwich. Rb\(^+\) forms a mixed sandwich when it is initially complexed to 15-Crown-5, but not when it is initially bound to 18-Crown-6. Cs\(^+\) readily forms mixed sandwiches, since it is too large to fit within either macrocycle.

Attempts to measure crown-cation bond energies using threshold CID have so far had limited success. The threshold for dissociation of the 18-Crown-6/K\(^+\) complex is tentatively 1.1 eV, corresponding to a bond energy of about 25 kcal/mol, which seems reasonable. Work is in progress to improve the reproducibility of these measurements.

\(^3\)Hancock, R. D. Acc. Chem. Res. 1990, 23, 253-257.

### Relative Sizes of Cations and Crown Ether Cavities

<table>
<thead>
<tr>
<th>Cation</th>
<th>Ionic radius(^1) Å</th>
<th>Crown ether</th>
<th>Cavity radius(^2) Å</th>
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</thead>
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<tr>
<td>Na(^+)</td>
<td>1.02</td>
<td>15-Crown-5</td>
<td>0.86-0.92</td>
</tr>
<tr>
<td>K(^+)</td>
<td>1.38</td>
<td>18-Crown-6</td>
<td>1.34-1.43</td>
</tr>
<tr>
<td>Rb(^+)</td>
<td>1.52</td>
<td>21-Crown-7</td>
<td>1.7</td>
</tr>
<tr>
<td>Cs(^+)</td>
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### Crown-Alkali Metal Reaction Summary

<table>
<thead>
<tr>
<th>Cation</th>
<th>15C5 → 18C6</th>
<th>18C6 → 15C5</th>
<th>15C5 “Sandwich”</th>
<th>18C6 “Sandwich”</th>
<th>15C5-18C6 “Sandwich”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>K(^+)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Rb(^+)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cs(^+)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
ION-MOLECULE REACTIONS OF GROUND AND EXCITED STATES
OF TRANSITION METAL IONS AND
THEIR RESULTING IONIC CLUSTER FRAGMENTS

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College Station, TX 77843

Atomic metal ions produced by electron impact ionization of transition metal carbonyls are formed
in both ground and metastable states and the distribution of states varies with the energy of the electron
beam\(^1\). Studies by various groups indicate that the state in which the reactant ion is produced can
significantly influence the chemistry of the ion-molecule reactions\(^2\).

This study focuses on the Fe\(^+\) system. Fe\(^+\) produced by EI of Fe(CO)\(_5\) reacts with neutral Fe(CO)\(_5\)
according to reactions 1-7. The charge transfer reactions (3-7) are endothermic for ground state Fe\(^+\) and
can only be a result of excited state Fe\(^+\). Generally, the relative intensities of the charge transfer products
increase from Fe(CO)\(^+\) (AP=11.52 eV) to Fe(CO)\(_5\)\(^+\) (IP=7.95 eV). The extent of electronic excitation can
be estimated from Table 1, and these data can be used to bracket the energy levels of the states giving
rise to charge transfer products.

The ligand exchange reactivity of Fe\(_2\)(CO)\(_3\)\(^+\) and Fe\(_2\)(CO)\(_4\)\(^+\) ionic cluster products formed by the
clustering reactions 1 and 2 are significantly different. For example, Fe\(_2\)(CO)\(_3\)\(^+\) readily undergoes ligand
exchange ion-molecule reactions with \(^{13}\)CO to displace all CO ligands; whereas only a fraction (ca. 40 %)
of the Fe\(_2\)(CO)\(_5\)\(^+\) ions are reactive toward ligand exchange\(^3\). The different reactivities of the Fe\(_2\)(CO)\(_5\)\(^+\) ionic
cluster fragment may be due to different structures for the Fe\(_2\)(CO)\(_3\)\(^+\) ions or electronic states of the
reactant Fe\(^+\) ions. If the Fe\(^+\) ions are formed with a distribution of long-lived electronically excited states,
the Fe\(_2\)(CO)\(_y\)\(^+\) ions may be formed with a distribution of internal energies.

\((\text{I})\) Clustering reactions

\[
\begin{align*}
\text{Fe}^{+} & + \text{Fe(CO)}_{5} \rightarrow \text{Fe}^{+} \rightarrow \text{Fe}^{+} + 2\text{CO} \\
\text{Fe}^{+} & + \text{Fe(CO)}_{6} \rightarrow \text{Fe}^{+} \rightarrow \text{Fe}^{+} \rightarrow \text{Fe}^{+} + \text{CO} \\
\end{align*}
\]

\((\text{II})\) Charge transfer reactions

\[
\begin{align*}
\text{Fe}^{+} & + \text{Fe(CO)}_{5} \rightarrow \text{Fe}^{+} \rightarrow \text{Fe}^{+} \rightarrow \text{Fe}^{+} + 2\text{CO} \\
\text{Fe}^{+} & + \text{Fe(CO)}_{6} \rightarrow \text{Fe}^{+} \rightarrow \text{Fe}^{+} \rightarrow \text{Fe}^{+} + 3\text{CO} \\
\text{Fe}^{+} & + \text{Fe(CO)}_{7} \rightarrow \text{Fe}^{+} \rightarrow \text{Fe}^{+} \rightarrow \text{Fe}^{+} + 4\text{CO} \\
\end{align*}
\]

These experiments were performed in a differentially pumped two-section ICR cell. For instance,
Fe\(^+\) is initially formed by EI of Fe(CO)\(_5\) (in region 1 of the two-section cell). Fe\(^+\) is isolated by using ion-
ejection techniques and partitioned to region two of the cell where it is allowed undergo collisions with
various reagents (CH\(_4\), CH\(_3\)OH, He, Ar, etc.). The Fe\(^+\) ion is then partitioned back to the reaction chamber
(region 1) and allowed to react with the neutral Fe(CO)\(_5\) for 100 milliseconds. The effects of collisional
quenching on the reaction chemistry of Fe\(^+\) are probed by monitoring the branching ratio [Fe\(_2\)(CO)\(_3\)\(^+\) / 
Fe\(_2\)(CO)\(_5\)\(^+\)] of the two ionic cluster products (reactions 1 and 2).

The collisional relaxation rates (Table 2) show nitric oxide as the most efficient quencher (3x10\(^{-9}\) 
cm\(^3\)/sec). Methanol and methane collisionally quench excited states of Fe\(^+\) at the same rate (2x10\(^{-9}\) 
cm\(^3\)/sec). Inert gases show interesting results; the heavier inert gases collisionally quench the excited
states of Fe\(^+\) at a faster rate than the lighter inert gases.

1610
Differences in the quenching rates for various reagents can be explained in terms of the energy transfer processes between excited Fe\(^*\) and each quenching reagent. For inert gases the energy transfer process involves electronic-to-translational energy transfer (E->T). The higher collisional relaxation probability of excited Fe\(^*\) by heavier Kr compared to the lighter He could be due to intramultiplet relaxation mechanism discussed by A. B. Caller\(^*\). On the other hand, electronically excited Fe\(^*\) can induce, electronic-to-vibrational, rotational and/or translational energy transfer to the other reagents (E->V, R, T).

The depletion of Fe\(_2\)(CO)\(_3\)* ionic cluster fragment over extended collisional relaxation time of Fe\(^*\) with NO, CH\(_3\)OH, CH\(_4\), N\(_2\), H\(_2\), D\(_2\), He, Ar, and Kr is a clear indication of the effect of electronic excitation on the ion-molecule reaction chemistry of Fe\(^*\) with the neutral Fe(CO)\(_8\) (reactions 1-7). The initial energy of the Fe\(^*\) ion can greatly influence the internal energy of the Fe\(_2\)(CO)\(_3\)* product ions.

Table 2. Rate constants (corrected for ionization gauge sensitivity\(^*\)) for collisional relaxation of Fe\(^*\) by various reagents.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Rate (cm(^3)/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>methane</td>
<td>2 x 10(^{-9})</td>
</tr>
<tr>
<td>methanol</td>
<td>2 x 10(^{-9})</td>
</tr>
<tr>
<td>nitric oxide</td>
<td>3 x 10(^{-9})</td>
</tr>
<tr>
<td>nitrogen</td>
<td>7 x 10(^{-10})</td>
</tr>
<tr>
<td>hydrogen</td>
<td>4 x 10(^{-10})</td>
</tr>
<tr>
<td>deuterium</td>
<td>3 x 10(^{-10})</td>
</tr>
<tr>
<td>helium</td>
<td>1 x 10(^{-10})</td>
</tr>
<tr>
<td>argon</td>
<td>5 x 10(^{-10})</td>
</tr>
<tr>
<td>krypton</td>
<td>6 x 10(^{-10})</td>
</tr>
</tbody>
</table>

Table 1. Ionization potentials of Fe\(^*\) electronic states and appearance potentials of the charge transfer products.

<table>
<thead>
<tr>
<th>state</th>
<th>configuration</th>
<th>ionization potential (eV)</th>
<th>appearance potential (eV)</th>
<th>charge transfer products</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^2)G</td>
<td>4s(^3)d(^4)</td>
<td>11.64</td>
<td>11.53</td>
<td>Fe(CO)*</td>
</tr>
<tr>
<td>(^2)F</td>
<td>4s(^3)d(^4)</td>
<td>11.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^2)H</td>
<td>4s(^3)d(^4)</td>
<td>11.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^2)P</td>
<td>4s(^3)d(^4)</td>
<td>11.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^4)G</td>
<td>4s(^3)d(^4)</td>
<td>11.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^6)S</td>
<td>4s(^3)d(^4)</td>
<td>10.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^4)F</td>
<td>4s(^3)d(^4)</td>
<td>10.68</td>
<td>10.68</td>
<td>Fe(CO)(_2)*</td>
</tr>
<tr>
<td>(^4)H</td>
<td>4s(^3)d(^4)</td>
<td>10.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^4)P</td>
<td>4s(^3)d(^4)</td>
<td>10.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^4)D</td>
<td>3d(^4)</td>
<td>10.42</td>
<td></td>
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</tr>
<tr>
<td>(^4)H</td>
<td>3d(^4)</td>
<td>10.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^4)P</td>
<td>3d(^4)</td>
<td>10.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^6)D</td>
<td>4s(^3)d(^4)</td>
<td>9.87</td>
<td>Fe(CO)(_3)*</td>
<td></td>
</tr>
<tr>
<td>(^4)F</td>
<td>3d(^4)</td>
<td>8.17</td>
<td>8.77</td>
<td>Fe(CO)(_4)*</td>
</tr>
<tr>
<td>(^6)D</td>
<td>4s(^3)d(^4)</td>
<td>7.92</td>
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</table>


Ionization Potentials and Reactivity of Coinage Metal Clusters

M.A. Cheeseman and J.R. Eyler
Department of Chemistry University of Florida
Gainesville, FL 32611-2046

The ionization potentials of several homoatomic and heteroatomic coinage metal clusters have been determined utilizing charge transfer bracketing and Fourier transform ion cyclotron resonance (FTICR) mass spectrometry. The clusters studied were Ag$_n$, Au$_n$, Cu$_m$, and Ag$_k$Cu$_l$, where n=2-3,5, m=2,3, and k and l = 1, 2. Atomic ionization potentials were also verified for each of the above metals as a test of the overall bracketing method. This work represents one of the first measurements of adiabatic ionization potentials for any of the above clusters. A number of additional reactions were observed between several of the charge transfer agents and metal cluster ions, including some which resulted in metal-metal bond cleavage.

Table I shows the bracketed ionization potentials and estimated uncertainties of those metal clusters produced with sufficient intensity to be studied by charge transfer reactions. Previous experimental and theoretical determinations of ionization potentials for these species are listed in columns three and four of Table I, respectively. The agreement between this work and previous experimental results is good, particularly with regard to those ionization potentials which are well known. There is also qualitative agreement with the theoretical treatments. Both of these results supports the viability of this technique for determining the ionization potentials of small metal clusters.

Additional side reactions which occurred between some of the charge transfer agents (CTAs) and the metal clusters, and resulted in metal-metal bond cleavage, were also studied. These have been analyzed thermochemically to yield lower limits for the bond dissociation energies of the metal-CTA ion/molecule complex. These estimates are presented in Table II. Also presented in Table II are bond dissociation energies of some Ag-CTA complexes determined by collision induced dissociation (CID). These values agree well with our other estimates.
TABLE I. Ionization Potentials (eV) of coinage metal clusters derived from charge transfer bracketing.

<table>
<thead>
<tr>
<th>Species</th>
<th>This Work</th>
<th>Literature</th>
<th>Theoretical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Experimental</td>
<td></td>
</tr>
<tr>
<td>Ag</td>
<td>7.61 ± 0.15</td>
<td>7.56, 6.4&lt;IP&lt;7.9</td>
<td>7.5</td>
</tr>
<tr>
<td>Ag</td>
<td>7.61 ± 0.15</td>
<td>7.56, 6.19, 6.4&lt;IP&lt;7.9</td>
<td>7.44, 8.03</td>
</tr>
<tr>
<td>Ag</td>
<td>&lt;7.00</td>
<td>&lt;7.00, 5.8&lt;IP&lt;6.4</td>
<td></td>
</tr>
<tr>
<td>Ag</td>
<td>7.27 ± 0.15</td>
<td>6.4&lt;IP&lt;7.9</td>
<td></td>
</tr>
<tr>
<td>Ag</td>
<td>7.27 ± 0.15</td>
<td>7.89, 7.46 ± .15</td>
<td>8.24</td>
</tr>
<tr>
<td>Cu</td>
<td>7.76 ± 0.10</td>
<td>7.72</td>
<td></td>
</tr>
<tr>
<td>Au</td>
<td>9.26 ± 0.10</td>
<td>9.22, 7.9&lt;IP</td>
<td>9.2</td>
</tr>
<tr>
<td>Au</td>
<td>9.18 ± 0.10</td>
<td>9.07, 9.08, 7.9&lt;IP</td>
<td>9.4</td>
</tr>
<tr>
<td>Au</td>
<td>7.27 ± 0.15</td>
<td>8.95, 6.4&lt;IP&lt;7.9</td>
<td>6.7</td>
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<tr>
<td>Au</td>
<td>7.61 ± 0.20</td>
<td>6.4&lt;IP&lt;7.9</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>7.76 ± 0.10</td>
<td>7.76, 6.14 ± 1.0</td>
<td>5.80..7.23</td>
</tr>
<tr>
<td>AgCu</td>
<td>7.61 ± 0.15</td>
<td>7.78</td>
<td></td>
</tr>
<tr>
<td>AgCu</td>
<td>7.46 ± 0.10</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>AgCu</td>
<td>7.27 ± 0.15</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

* Determined by CID
~ This work

Table II. Bond energies(eV) of coinage metal dimers derived from second and third law calculations along with bond strengths of metal-CTA complexes derived from thermochemical relationships and by CID studies.

<table>
<thead>
<tr>
<th>Species</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>1.66</td>
</tr>
<tr>
<td>Ag*</td>
<td>1.66 ± .04</td>
</tr>
<tr>
<td>AgCu</td>
<td>1.76</td>
</tr>
<tr>
<td>Cu</td>
<td>2.03</td>
</tr>
<tr>
<td>Au</td>
<td>2.29</td>
</tr>
<tr>
<td>Ag(DMAN)*</td>
<td>1.67 ± .12</td>
</tr>
<tr>
<td>Ag(mTOL)*</td>
<td>1.71 ± .23</td>
</tr>
<tr>
<td>Ag(AZ)*</td>
<td>1.61 ± .27</td>
</tr>
<tr>
<td>Ag(DMAN)*</td>
<td>&gt; 1.66 ~</td>
</tr>
<tr>
<td>Ag(mTOL)*</td>
<td>&gt; 1.66 ~</td>
</tr>
<tr>
<td>Ag(AZ)*</td>
<td>&gt; 1.66 ~</td>
</tr>
<tr>
<td>Ag(DEAN)*</td>
<td>&gt; 1.66 ~</td>
</tr>
<tr>
<td>Au(MDCBZ)*</td>
<td>&gt; 2.29 ~</td>
</tr>
<tr>
<td>Au(BZ)*</td>
<td>&gt; 2.29 ~</td>
</tr>
<tr>
<td>Au(FBZ)*</td>
<td>&gt; 2.29 ~</td>
</tr>
<tr>
<td>Cu(AZ)*</td>
<td>&gt; 2.03 ~</td>
</tr>
<tr>
<td>Cu(DMAN)*</td>
<td>&gt; 2.03 ~</td>
</tr>
<tr>
<td>Cu(mTOL)*</td>
<td>&gt; 2.03 ~</td>
</tr>
<tr>
<td>Cu(AN)*</td>
<td>&gt; 2.03 ~</td>
</tr>
</tbody>
</table>
Reactions of Laser Desorbed Metal Ions With Benzene
Steven L. VanOrden, Brian T. Cooper, R. Marshall Pope, Steven W. Buckner, Department of Chemistry, University of Arizona, Tucson, AZ, 85721

Introduction
This work involves the reactivity of early transition metal cations (Y*, La*, Nb*, and Ta*) with benzene. These metal cations have been previously observed to be highly reactive toward hydrocarbons, exhibiting predominantly C-H insertion mechanisms. We have studied the product distributions, kinetic isotope effects, and subsequent reactions for these metal cations. It is found that these metals are highly reactive toward benzene, and that cleavage of the benzene ring competes with formation of the metal-benzyne complex by dehydrogenation.

One of the problems associated with performing laser desorption/ionization and photochemistry experiments with a pulsed Nd:YAG laser coupled to a Fourier transform ion cyclotron resonance spectroscopy is timing the laser and MS sequences. We have designed a new interface that coordinates the laser and mass spectrometer. This interface was used for the present laser desorption experiments.

Experimental
All experiments were performed on a Fourier transform ion cyclotron resonance mass spectrometer. Atomic metal cations were generated by focussing the fundamental output of a Spectra-Physics DCR-11-3 pulsed Nd:YAG laser (pulse energies of ~135 mJ) on pure metal blocks. Benzene pressure in the vacuum chamber was ~5x10^-8 torr (uncorrected). After initial ionization, the metal ions were trapped for 200-400 msec to allow for collisional and radiative cooling. After the cooling period the ions were isolated by swept double resonance ejection pulses. This cooling period should allow for nearly complete translational cooling (35-70 collisions). However, there is evidence that a small portion of the remaining ions might have some electronic excitation after this period.

Results
All four metals investigated were found to dehydrogenate at least one benzene to form a metal-benzyne complex. Scheme 1 shows the reactive pathways observed for each of the metal species. The relative branching ratios, listed for C,H6, are found to be very similar to those of C,D6. Only a small kinetic isotope effect is observed (~1.1) which could indicate that the dehydrogenation involves a tight transition state. The observed charge transfer reactions to form C,H7+ are endothermic and are probably due to incomplete collisional cooling of the metal ions. At very long reaction times it was found that 4 benzenes were dehydrogenated to form Ta(C,H5)+, but the reaction could be driven no further. The observation of C,H4 and C,H ligands for Nb* and Ta+ is the first observation of ring cleavage of benzene by transition metal cations. The lack of subsequent reaction by the Y-benzyne and La-benzyne complexes could offer insight into the mode of bonding. Consider structures I and II. For structure I both valence electrons are used in bonding and we expect little or no secondary reactions. A π-bonded benzyne such as in structure II, however, would still be capable of further reaction.
SCHEME 1

La$^+$ + \[\text{C}_2\text{H}_4\] $\rightarrow$ La(C$_4$H$_4$)$^+$ + H$_2$

Y$^+$ + \[\text{C}_2\text{H}_4\] $\rightarrow$ Y(C$_4$H$_4$)$^+$ + H$_2$

Nb$^+$ + \[\text{C}_2\text{H}_4\] $\rightarrow$ Nb(C$_4$H$_4$)$^+$ + H$_2$

Ta$^+$ + \[\text{C}_2\text{H}_4\] $\rightarrow$ Ta(C$_4$H$_4$)$^+$ + H$_2$

I \[\text{Y} \equiv \text{O} \quad \text{II} \quad \text{Y} \equiv \text{R}]

* Product Indicates Excited Metal Ion
INTRODUCTION

The photochemically induced polymerization of epoxides by cyclopentadienyl iron arene complexes is a commercially significant reaction used for photolithography. The FeCp* cation is thought to be a key intermediate in the reaction (1,2). We have previously investigated the gas phase ion chemistry of FeCp* with ethylene oxide, and with other cyclic ethers, to learn details of the mechanism of this reaction (3). We have now extended these studies to the reactions of cyclic ethers with other first row transition metal cyclopentadienyl cations, such as CoCp* and NiCp*. In addition, the effect of internal energy of the FeCp* ion on its reactivity with ethylene oxide has been investigated.

EXPERIMENTAL

The experiments were performed using a Fourier transform mass spectrometer with a 1-3/4" cubic cell, a 1 Tesla electromagnet and an Ionspec Omega data system. The vacuum system is pumped by a 330 l/s turbomolecular pump to a base pressure of 2x10⁻¹⁰ Torr. The vacuum system is equipped with two pulsed valves and a sapphire sealed leak valve for introducing volatile samples. CoCp*, NiCp*, and FeCp* were made by electron ionization of cobaltocene, nickelocene, and ferroocene introduced through a pulsed valve, opened for 10 ms. The metallocene pressure peaks 100 ms after the pulsed valve is opened, producing a maximum pressure of ca. 10⁻⁶ Torr, and fall to less than 10⁻⁸ Torr within 1 s. FeCp* was also formed by charge exchange ionization of ferroocene as described below.

FeCp* internal energy was varied using three methods (1) Adjusting the electron ionization energy between 20 eV and 70 eV demonstrated strong control on the observed reactivity of the FeCp* ion. (2) The internal energy of FeCp* formed by electron ionization was reduced by collisions with background ferroocene. The number of collisions a FeCp* ion experienced was controlled by changing the delay time of electron beam with respect to the pulsed valve event in which ferroocene was introduced. Short delay times produce cool FeCp*, because several collisions can occur between the FeCp* ions and the ferroocene vapor before it is pumped from the vacuum system. Increasing the delay between the pulsed valve introduction of the metallocene and the electron beam event yield ions with more internal energy. (3) Charge exchange ionization of ferroocene allows control of the energy deposited into the ions that are formed, through the selection of the appropriate reagent ion. As the recombination energy of the reagent ion increases, the amount of internal energy present in the FeCp* ion increases, for the reaction shown below.

\[
R^* + \text{FeCp}_2^+ \rightarrow R + \text{FeCp}_2^* \rightarrow \text{FeCp}_2^* + \text{Cp}^-
\]

RESULTS

The sole reaction between ethylene oxide and CoCp* or NiCp* is charge exchange. The odd electron ethylene oxide undergoes reactions with neutral ethylene oxide to form a protonated molecule, and other higher mass species. In contrast, FeCp* abstracts oxygen from ethylene oxide to form FeCpO*, and also undergoes charge exchange with the cyclic ether. However, CoCp* and NiCp* show the same reactivity as FeCp* toward the larger cyclic ethers, oxetane and tetrahydrofuran. The observed reaction for the latter ethers is a condensation of the ion and ether neutral. Also, CoCp* and NiCp* behave like FeCp* in reactions with ethylene sulfide. The abstraction of sulfur from one or two molecules of ethylene sulfide to form MCpS* and MCpS₂* is observed for the reactions of all of these cyclopentadienyl metal cations.
The two competing reaction channels of FeCp* with ethylene oxide, oxygen abstraction and charge exchange, can be individually enhanced by controlling the internal energy of the metal containing ion. With all three methods of changing FeCp+ internal energy, the same trend is observed. When the internal energy of FeCp* is increased, the ratio of oxygen abstraction to charge exchange is found to increase. At low internal energy, charge exchange is the major product, while at high energy, oxygen abstraction is the sole reaction, as shown in Figure 1.

REFERENCES


Figure 1. Mass spectra of the products of the reaction of ethylene oxide with FeCp* formed by 70 eV electron ionization of ferrocene admitted through a pulsed valve, delaying the electron beam (a) 80 ms and (b) 660 ms after the ferrocene is pulsed into the vacuum system. The C\textsubscript{2}H\textsubscript{4}OH* ion found in the top mass spectrum results from the odd electron molecular ion of ethylene oxide, formed by charge exchange, reacting with a second molecule of ethylene oxide.
ION-MOLECULE REACTIONS OF IRON PORPHYRINS WITH NO$_2$

Henglong Chen, T. E. Hagan, S. E. Groh and D. P. Ridge*
Department of Chemistry and Biochemistry and Center for Catalytic Science and Technology, University of Delaware, Newark, DE19716.

O atom transfer reactions between NO$_2$ and iron porphyrins in the gas phase have been observed. The neutral species 1, 2, 3 and 4 were introduced into the vacuum chamber of a Fourier transform ion cyclotron resonance (FT-ICR) spectrometer (FTMS-2000 Extrel, Madison, WI) at pressures on the order 10$^{-8}$ torr. The molecular anions of these species were formed by electron attachment and molecular cations by electron impact. NO$_2$ was introduced through batch inlet at a pressure of ca. 10$^{-7}$ torr. The anions of 3 and 4 (M) were found to react according to (1) and (2).

\[ k_1 \]

\[ M^- + NO_2 \rightarrow M(0^-) + NO \] (1)

\[ k_2 \]

\[ ------\rightarrow M(NO_2)^- \rightarrow M(NO_2)_{2}^- \] (2)

The variation of reactant and product ions with time are shown in Fig. 1. Also observed are M(NO$_3$)$^-$ and M(NO$_2$)(NO$_3$)$^-$. at small steady state concentrations. They are probably intermediates in the conversion of M(O)$^-$ to M(NO$_2$)$_2$$. (k$^1 + k^2) is about 2.3 \times 10^{-10}$ cm$^3$ s$^{-1}$

A two cell experiment where M$^-$ was prepared in one cell and then transferred to a second differentially pumped cell where it is exposed to NO$_2$ indicated that reaction (1) is promoted by internal energy in the reactant ion. Since M$^-$ is formed by electron attachment, its initial internal energy is the electron affinity of M. As the time that M$^-$ is stored in the first cell is increased allowing M$^-$ to cool, the relative amount of M(O)$^-$ observed in the second cell is diminished. CID of M(NO$_2$)$^-$ gives only M$^-$ and M(O)$^-$ as ionic fragments. These results suggest that Fe-O bonding is at least accessible to the adducts of anions of 3 and 4 with NO$_2$ in the gas phase.

On the other hand, 1 and 2 are not observed to extract O atom from NO$_2$. Instead charge transfer is the major process. This suggests that the chemistry of 3 and 4 is promoted by the ortho amido substituents on the porphyrin.

The reactions of the cation of 2 (M) with NO$_2$ are illustrated in Fig. 2. The products of this reaction are M(OH)$^+$ and M(NO)$^+$. The formation of M(OH)$^+$ must proceed by the intermediacy of either MH$^+$ or M(O)$^+$. A small amount of MH$^+$ is observed (the M+1 mass peak slightly exceeds the expected $^{13}$C peak). In the absence of NO$_2$, however, M$^+$ is undepleted and MH$^+$ does not grow at long reaction time. This suggests that M(OH)$^+$ results from reaction (3).

\[ \text{NO}_2 + \text{RH} \rightarrow \text{M}^+ \rightarrow \text{M(OH)}^+ \rightarrow \text{M(OH)}^+ \] (3)

M(OD)$^+$ was not observed in the presence of D$_2$O, (CD$_3$)$_2$C=O and C$_6$D$_6$ indicating that RH in reaction (3) is the neutral porphyrin. The resulting R$^-$ is a very stable radical.
The formation of M(NO)$^+$ in single bimolecular encounter between M$^+$ and NO$_2$ requires loss of an O atom. A free O atom is a most unstable leaving group. It is also observed that the ratio of M(OH)$^+$/ M(NO)$^+$ is proportional to the ratio of the pressures of RH and NO$_2$. This was tested in two kinds of experiments. In one experiment, the ions were formed by electron impact, the NO$_2$ pressure varied by controlling the batch inlet pressure and the RH pressure varied by controlling the probe temperature. In a second experiment, the M$^+$ ion was formed by laser desorption and M(OH)$^+$ is formed only at short delays after the laser pulse when desorbed neutral RH is present. M(NO)$^+$ is the only product at long delays after the laser pulse. Both of these suggest that M(NO)$^+$ is formed by the two step reaction (4).

$$\begin{align*}
\text{NO}_2 & \rightarrow \text{NO} \\
\text{M}^+ & \rightarrow \text{M(OH)}^+ \rightarrow \text{M(NO)}^+ \\
\text{-NO} & \text{-O}_2 \\
\end{align*}$$

Cations of 1 and 3 react with NO$_2$ to give the same products as 2. The cation of 4 does not react with NO$_2$. The conformation of cation of 4 has been characterized in the gas phase and the "tethered" imidazole base is found to be coordinated with the metal$^{[1]}$. The failure of the cation of 4 to react with NO$_2$ thus suggests that O atom transfer from NO$_2$ requires a coordinatively unsaturated metal (13 valence electrons) as is found in the cations of 1, 2 and 3.


---

Fig. 1

![Fig. 1](image1)

**PORPHYRINS STUDIED:**

- 1. \(R=X=Y=H\)
- 2. \(R=X=Y=F\)
- 3. \(R=H, X=Y=\text{C}6\text{H}5\)
- 4. \(R=X=H, Y=\text{C}6\text{H}5\)

Fig. 2

![Fig. 2](image2)
Reactions and Thermochemistry of Molecular Cluster Ions

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Introduction
Protonated cluster ions can be generated following the ionization of neutral clusters containing protic hydrogens and lone-pair electron donor sites. In these systems, hydrogen-bonded networks can be extended indefinitely. The relation between energetics and distributions is of interest because ion cluster formation usually involves ionization with excess energy, often followed by strongly exothermic intra-cluster reactions, while the cluster binding energies are relatively small. In this paper, we report the results of a joint beam expansion/thermochemical study of blocked hydrogen bonding in acetonitrile - methanol binary clusters.

Experimental
Acetonitrile-methanol binary clusters, generated by pulsed adiabatic expansion in a supersonic cluster beam apparatus, were ionized by EI and subsequently mass analyzed in a quadrupole mass spectrometer. Equilibrium measurements were carried out using the NIST pulsed high-pressure mass spectrometer.

Results
Figure 1 displays a typical 70 eV EI mass spectrum observed from the ionization of a cluster beam generated from the expansion of a 1:1 vapor mixture of CH₃CN and CH₃OH in He carrier gas. The prominent peaks in the mass spectrum are due to the sequence H⁺MnA₂ (denoted as aₙ) where A and M are acetonitrile and methanol, respectively. This manifold is predominant over a wide range of vapor composition (30-80% A with respect to M) and stagnation pressures (1-6 Bar) and is strongly independent of the electron impact energy (20-100 eV), indicating that the cluster ions H⁺MnA₂ have a particularly stable structure independent of the distribution of the neutral clusters produced. In order to define the thermochemistry associated with the cluster sequences, we measured the enthalpies of the various clustering and exchange equilibria. The results for clusters containing up to four molecules are shown in Figure 2. The results show that the switching of CH₃CN for CH₃OH is favorable until the clusters incorporate two CH₃CN molecules and become blocked at both ends of the CH₃OH chain. Past this, further exchange becomes endothermic. In conclusion, the cluster ion distributions from beam expansion are sensitive to thermochemical differences as small as 1-3 kcal/mol. The sensitivity to thermochemistry is observed even when ionization and intra-cluster proton transfer can deposit large amounts of energy. This suggests that the final evaporation steps occur from clusters with little excess energy. Thermochemical data make it possible to correlate the cluster distributions with energetics, and will be useful also in calculating the rate constants for evaporation processes.
Figure 1

Figure 2: $\Delta H^O$ (kcal/mol) for clustering and exchange equilibria.
"LADDER" Algorithms for Constructing Equilibrium Affinity Scales

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Dept. of Chemistry, University of Tennessee, Knoxville TN 37996-1600

Many equilibrium affinity scales have thus far appeared in the literature, such as gas phase basicity and acidity scales. Creation and ordering of these scales from the original equilibria is tedious and somewhat arbitrary when done by hand. We previously reported a program that creates the scale, gives the best fitting absolute AG's for each member, and graphs the scale. We now report significant improvements to the program.

Prior to data analysis, the program discards pendant values (those linked to only one other compound) and breaks the data into fusion subsets, which are analyzed separately. The latter are defined as sections of the "ladder", bounded by compounds on which all equilibria values from either higher or lower in the ladder terminate. Both of these actions decrease computational time in data analysis, because computational time is a power function of the number of bases. The data can also be analyzed using a number of weighting schemes. A scheme with even weighting is best when unusual chemistry is not expected. If a wide range of AG values are present in the data, a Gaussian weighted fit can also be considered. The Gaussian fit is chemically reasonable since $K_{eq}$ is often not known to better than ±10%. While a measured AG < 1.0 kcal/mol is probably known ± 0.1, for a 3.0 kcal/mol process, the error is more likely ±0.5 kcal/mol.

Mathematically the problem is a slightly overdetermined one: typically the number of measurements is somewhat less or comparable to twice the number of compounds. The difficulty in assignment of the best fit lies in recognizing outliers in the measurement data. These outliers could be due to experimental error or to unusual chemistry. Unnoted, the outlying data may skew the solution that best fits the data.

Three different methods of analysis are used: least squares/sums, iterative smoothing, and simplex. For all three, the equilibrium pairs are first ordered as a matrix of all possible pairs, with the values for the measured energies entered at the appropriate points. For the first method, this matrix is then factored via Householder transformation with pivoting, and the least squares solution is obtained. The method gives a rapid solution. In addition, the same factored matrix can be re-used many times to test the effects of changing a questionable AG value. The disadvantage of the method is in predicting outliers. In the simple least squares method, true outliers can be missed, while good measurements can be indicated to be outliers.

The second method of data analysis, iterative smoothing, treats the data as a planar projection of the matrix and then tries to fit a smooth plane to the data. The method is best for predicting small AG's between nearby compounds that correspond to the smoothed gradient. Its disadvantages are that the solution is dependent on the ordering of the pairs and no weighting of measurements is possible.
The third method is primarily concerned with detecting the true outliers. The simplex method is used in conjunction with least trimmed squares. The squares of the residuals are first ordered, then the largest of these are discarded from the minimization function. This method is best for predicting outlying data, but is also time intensive. As long as the three solution qualitatively agree, the least squares routine is accepted as the best fit, and the simplex method as the method for detecting questionable data.

The program then splices the fusion sets together, reinserts the pendant measurements, and anchor the scale to the accepted AG for one base. Another short program graphs the scale.

We define three general classes of ladders. Pendant ladders involve many pendant measurements or a whole series of pendants. These ladders are only barely overdetermined (number of equilibria approaching the number of compounds) and the program cannot really help. Proper construction of a ladder requires two or more pathways between any two compounds. A ladder rich in multiple pathways can consist of a number of fusion sets, or be one fusion set in its entirety. The former are easier to calculate a fit for, but the latter are mathematically the best ladders. For a multiple fusion set ladder, once the outlying data has been re-investigated, complete scales may be formed by spanning the fusion set boundaries. The program runs on PCs, but a large scale (more than 70 compounds and 150 measurements) may require the use of a mainframe computer.

The program has been used to isolate problems in existing scales, can be used in the creation of new ones, and is available from the authors.

A small sample scale:

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
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<td>0.3</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e</td>
<td></td>
<td></td>
<td>5.77 (pendant)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td></td>
<td></td>
<td></td>
<td>5.63</td>
<td></td>
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<tr>
<td>c</td>
<td>0.1</td>
<td>0.4</td>
<td>5.37 (fusion)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.23</td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>0.2</td>
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<td></td>
<td>5.00</td>
</tr>
</tbody>
</table>
The Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometer has been used to determine many gas phase physical quantities such as relative and absolute ion/molecule reaction rate constants, proton and electron affinities, and ionization potentials. Measurement (or at least an estimate) of the kinetic energy of ions in an FTICR instrument is important since any or all of the above mentioned quantities may be dependent on kinetic energy.

The kinetic energy of ions produced in an FTICR mass spectrometer has been estimated by two different means in this work. One method utilized kinetic energy dependent ion/molecule reactions and, by comparison with previous work, resulted in an ion kinetic energy. Ion kinetic energy is related to ion temperature by the equipartition theorem. The other method, based on thermodynamics, estimated ion temperatures using proton transfer equilibrium measurements on molecules for which standard free energies of proton transfer are known.

Investigations involving three different systems (two kinetic, one thermodynamic) are reported here. For all data presented here, a relaxation delay after ion formation and prior to the beginning of the data acquisition period was implemented to allow ions formed near the trapping plates time to be collisionally relaxed to the center of the cell where ion detection is the most efficient. The first ion/molecule reaction system used was \( \text{O}_2^+ + \text{CH}_4 \rightarrow \text{CH}_3\text{O}_2^+ + \text{H} \). Data obtained here resulted in a rate constant of \( 9.4 \pm 0.4 \times 10^{-13} \text{ cm}^3\text{s}^{-1} \). This value corresponds\(^1\) to an average ion temperature of 1600 K. The ion/molecule reaction studied most fully was \( \text{Ar}^+ + \text{N}_2 \rightarrow \text{Ar} + \text{N}_2^+ \). It was found to have a rate constant of \( 2.4 \pm 0.2 \times 10^{-11} \text{ cm}^3\text{s}^{-1} \) which indicates\(^2\) an average temperature of 690 ± 190 K. This reaction was also studied as a function of pressure and trapping voltage. The results of these experiments are shown in Figures 1 and 2. The final system examined in this work was the proton transfer equilibrium between toluene and ethyl benzene. Measurement of the equilibrium constant in conjunction with a known standard free energy change\(^3\) for this reaction of 1.0 ± 0.5 kcal/mol provided a temperature estimate. The equilibrium constant, which is independent of the chemical ionization reagent and total pressure over the range studied, was found to be 0.203 ± 0.022. The temperature indicated by this "thermometer" is 316 ± 160 K. A summary of the temperatures found and the pressures studied with each of the three systems is given in Figure 3.

None of the temperatures obtained from the ion "thermometers" presented here agree. One might conclude based on the disagreement that the measurement of ion kinetic energy through chemical reactions is not feasible. However, even though an absolute temperature still seems uncertain, some definite conclusions regarding ion kinetic energy can be made. From the \( \text{Ar}^+ + \text{N}_2 \) study two statements can be made. First, \( \text{Ar}^+ \) ions, if formed with excess kinetic energy, are collisionally relaxed to some temperature that additional collisions will not reduce. This occurs with an average of as few as 13 collisions (based on a Langevin cross-section). Since no effect of total pressure is observed on this base kinetic energy, one might conclude that during the relaxation period (a 100 ms delay after ion formation and prior to data acquisition) the excess z-kinetic energy is to a large extent removed rather than merely transformed into x- and y- kinetic energy. Finally, even if the ions are translationally excited by the trapping potential well, the same base kinetic energy is reached for all trapping voltages between 0.3 and 5 volts with 40 collisions or less.

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**Figure 1**

$k$ vs Pressure

![Graph showing $k$ vs Pressure](image)

**Figure 2**

$k$ vs Trapping Voltage

$P_{(tot)} = 1.2 \times 10^{-8}$ torr

![Graph showing $k$ vs Trapping Voltage](image)

**Figure 3**

Ion Temperature Summary

<table>
<thead>
<tr>
<th>System</th>
<th>Pressure Range</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>$O_2^+ + CH_4 \rightarrow CH_2O$ + H</td>
<td>$10 \times 10^{-6}$ torr</td>
<td>1600 K</td>
</tr>
<tr>
<td>$Ar^+ + N_2 \rightarrow Ar + N_2^+$</td>
<td>$0.4 - 20 \times 10^{-6}$ torr</td>
<td>980 - 1900 K</td>
</tr>
<tr>
<td>Proton Transfer Eq.</td>
<td>$2.8 - 94 \times 10^{-6}$ torr</td>
<td>318 - 190 K</td>
</tr>
</tbody>
</table>

1625
At low pressure in the FT-ICR, association reactions of Si(CH$_3$)$_3$$^+$ with a number of species proceed by a radiative stabilization mechanism$^1$. The experimental efficiencies, $\Phi_{(exp.)}$ ($\Phi_{(exp.)} = k_a/k_c$, where $k_a$ is a bimolecular rate constant and $k_c$ is a collisional rate constant) measured at 300K are in reasonable agreement with Dunbar's theoretical values derived from RRKM theory$^2$. The model predicts that the radiative association efficiency depends on the bond dissociation energy, $E_b$, the temperature, $T$, and number of vibrational degrees of freedom, $N$. We examine here the effects of temperature and bond energy on the association reactions of Si(CH$_3$)$_3$$^+$. In order to broaden our study of association reactions, one other system, the formation of acetone proton-bound dimer, was also studied.

A number of oxygen-, sulfur- and aromatic compounds have been studied with Si(CH$_3$)$_3$$^+$. As shown in Fig. 1, all radiative association efficiencies decrease with increasing temperature over the range T = 300K to 420K (two species, acetone and dimethylether adducts, have been measured up to 510K), in qualitative agreement with theory. The slope of the plot of Ln($\Phi_{(exp.)}$) against $T$ gives the temperature coefficient, $\phi\prime$. $\phi\prime$ increases linearly with $E_b$ as shown in Fig. 2. The experimental efficiency, $\Phi_{(exp.)}$, increases with $E_b$, so Fig.2 indicates that highly efficient association reactions have lower temperature coefficient than less efficient association reactions. For species studied here, $\phi\prime$ of furan is 10 times larger than $\phi\prime$ of the c-hexanone adduct. The $\phi\prime$ of acetone proton-bound dimer does not fall on the line in the plot of $\phi\prime$ vs. $E_b$, which indicates that $\phi\prime$ varies with factors other than $E_b$.

Apparent bimolecular rate constants $k_a$ in slow association reactions ($\Phi_{(exp.)} \ll 1$) are pressure dependent. From a kinetic analysis$^3$ of plots of $k_a$ vs. reactant neutral pressure, one can get the dissociation rate constant $k_b$ and the radiative rate constant $k_r$ experimentally. In the case of the acetone proton-bound dimer (Table1), $k_b(\text{exp.})$ agrees well with $k_b(\text{theo.})$ from RRKM theory, and $k_r$ is nearly constant in the measured temperature range.

As the above studies have shown, the bond energy $E_b$ has a crucial effect on $k_b$ and consequently on the association efficiency. Bond energies from equilibrium constant measurement are listed in Table 2. Comparison of $E_b$ with P.A. and I.P. shows that $E_b$ increases linearly with increasing P.A. and decreasing with I.P. for compounds with similar functional groups. A difference is noted in behaviour between what we call closed aromatic hydrocarbons (at least one six-membered ring without H substituents) and open aromatic hydrocarbons (all others).

References

Table 1: Determination of $k_2$ and $k_3$ for acetone proton-bound dimer

<table>
<thead>
<tr>
<th>T(K)</th>
<th>$k_2(s^{-1})$</th>
<th>$k_{k_{mol}} \times 10^4(s^{-1})$</th>
<th>$k_{k_{mol}} \times 10^4(s^{-1})$</th>
<th>$k_{k_{mol}} \times 10^4(s^{-1})$</th>
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<tr>
<td>300</td>
<td>98</td>
<td>2.4</td>
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<td>330</td>
<td>121</td>
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<td>360</td>
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<td>29.7</td>
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<tr>
<td>420</td>
<td>157</td>
<td>48.2</td>
<td>33.0</td>
<td>11.0</td>
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</table>

Table 2: Correlation of $E_b$ with I.P. and P.A.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>$E_b$</th>
<th>P.A.</th>
<th>I.P.</th>
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<td>Furan's</td>
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<tr>
<td>Furan</td>
<td>159</td>
<td>803</td>
<td>857</td>
</tr>
<tr>
<td>Methylfuran</td>
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</tr>
<tr>
<td>Dimethylfuran</td>
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<td>874</td>
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<td>Thiophen's</td>
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<tr>
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<td>821</td>
<td>856</td>
</tr>
<tr>
<td>Benzothiophene</td>
<td>176</td>
<td>*</td>
<td>784</td>
</tr>
<tr>
<td>Dibenzothiophene</td>
<td>179</td>
<td>*</td>
<td>762</td>
</tr>
<tr>
<td>Benzene</td>
<td>100</td>
<td>758</td>
<td>893</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>157</td>
<td>814</td>
<td>785</td>
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<td>PAH's</td>
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<td>Phanthrene</td>
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</tr>
<tr>
<td>PAH's</td>
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<td></td>
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<tr>
<td>1,2,3,4-Dibenzenanthracene</td>
<td>182</td>
<td>850</td>
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<tr>
<td>Perylene</td>
<td>187</td>
<td>884</td>
<td>666</td>
</tr>
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</table>

Fig. 2: Acetone proton-bound dimer

Fig. 1: Various compounds in the mass spectrometry study.

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The O* ion produced by solar ultraviolet photoionization of Atomic oxygen is the major ionic species in the ionosphere. Since the rates of radiative recombination of O* with electrons are several order of magnitude smaller than those for dissociative recombination of \( N^*, O^*, NO^* \) and \( CO_2^* \) with electron, the charge transfer reactions which convert O* into \( N^*, O^*, NO^* \) and \( CO_2^* \) constitute the most important ion-molecule reactions in the ionosphere.

Using a tandem photoionization mass spectrometer, we have measured absolute total cross sections for the following reactions:\(^1\)^\(^2\)

\[
\begin{align*}
O^*(^4S) + H_2 &\rightarrow O + H_2^* \quad (1) \\
&\rightarrow O + H + H^* \quad (2) \\
O^*(^4S) + N_2 &\rightarrow N + NO^* \quad (3) \\
&\rightarrow O + N_2^* \quad (4) \\
&\rightarrow O + N + N^* \quad (5) \\
O^*(^4S) + CO_2 &\rightarrow O + CO_2^* \quad (6) \\
&\rightarrow O + O + CO^* \quad (7) \\
&\rightarrow CO + O_2^* \quad (8) \\
&\rightarrow O + O_2 \text{ (or } O + O) + C^* \quad (9)
\end{align*}
\]

Preliminary results on the corresponding reactions involving excited \( O^*(^2D) \) and \( O^*(^2P) \) have also been obtained. Charge transfer is found to be overwhelmingly the major channel for reactions of excited \( O^*(^2D, ^2P) \).

References:


(2) G. D. Flesch and C. Y. Ng, J. Geophys. Res. to be submitted.
GAS PHASE REACTIONS OF CF+, CF2+, and CF3+ WITH HALOCARBONS
Robert A. Morris, J. M. Van Doren, A. A. Viggiano, and John F. Paulson
Phillips Laboratory, Geophysics Directorate/LID, Hanscom AFB, MA 01731-5000

Ion-molecule reactions occurring in plasmas containing halocarbons have received little attention from experimenters. In this abstract we report results for the gas phase reactions of CF+, CF2+, and CF3+ with the halocarbons CF3Cl, CF3Br, CF3I, CF4, and C2F6. The investigation was conducted using a variable temperature-selected ion flow tube (VT-SIFT) instrument operated at 300 and 496 K. Few of these reactions have been studied previously. None has been investigated by the SIFT technique or as a function of temperature.

The ion CF+ reacts rapidly with CF3X (X = Cl, Br, I) producing the ions CF2X+. In the case of CF3Cl, CF3+ is also produced as a minor product. Rate constants for these and the other reactions studied are given in Table 1. Curvature was observed in the pseudo-first-order kinetics plots (logarithm of CF+ signal vs. reactant neutral flow rate) for the reactions of CF+ with CF4 and C2F6. In both cases the curvature is attributed to the presence of two CF+ states (vibrational or electronic) of differing reactivities toward the perfluorocarbon of interest. This conclusion is supported by our observation of efficient charge transfer from CF+ to NO, a reaction which is endothermic 3 kJ/mole for the ground state of CF+. For the reaction of CF+ with NO, we again observed curvature in the kinetics plots. Rate constants for the slow reacting state, kslow, and for the sum of reaction and quenching of the fast reacting state (kfast + kq) were obtained by fitting a double exponential function to the data. The values of kslow are 1.1 x 10^-11 and 4.2 x 10^-11 cm^3 s^-1 at 300 and 496 K, respectively. These values are assumed to correspond to the ground state of CF+. The activation energy derived from these data is 8 kJ/mole. The values of (kfast + kq) are 8.7 x 10^-10 and 6.8 x 10^-10 cm^3 s^-1 at 300 and 496 K, respectively, and are equal to the collisional rate constants within experimental uncertainty.

The reactions of CF2+ with CF3X yield CF3+ and CF2X+ for X = Cl and Br; for X = I, CF2I+ and CF3I+ are produced. Production of CF3X+ is exothermic for X = I only. The overall reactions proceed at the collision rate at 300 and 496 K, and the branching ratios are not strongly dependent on temperature. The reactions of CF2+ with CF4 and C2F6 produce CF3+ and C2F5+, respectively. The rate constants decrease significantly with increasing temperature. In the reaction of CF2+ with CF3X, the products CF3+ + CF2X (X = F, Cl, Br) may arise from transfer of either F or X. For the products CF2X+ + CF3 (X = F, Cl, Br, I), transfer of either X or F- may pertain. Similarly, the C2F5+ product of the reaction between CF2+ and C2F6 may arise from transfer of either F- or CF3. Experiments in which the reactant ion is 13CF2+ are planned with the hope of elucidating the reaction mechanism(s).

CF3+ reacts with CF2X (X = Cl, Br, I) via fluoride transfer producing CF2X+ as the only ionic product for all X. The rate constants, which are below the collisional values at both 300 and 496 K, decrease sharply with increasing temperature and increase for the heavier halides. These results are consistent with the reactions occurring on a double-well potential.
surface. The measured negative temperature dependences are: \( k(X = \text{Cl}) \sim T^{-1.9}; k(X = \text{Br}) \sim T^{-2.0}; k(X = \text{I}) \sim T^{-1.4} \). No reaction was observed for the \( \text{CF}_3^+ / \text{CF}_4 \) system. \( \text{CF}_3^+ \) appeared to react very slowly with \( \text{C}_2\text{F}_6 \) at 300 K, producing \( \text{C}_2\text{F}_5^+ \), but a reaction with an impurity in the \( \text{C}_2\text{F}_6 \) cannot be ruled out. No reaction was observed at 496 K.

1 Air Force Geophysics Scholar.
2 Formerly Air Force Geophysics Laboratory.

Table 1. Ionic products and rate constants for the title reactions measured at 300 and 496 K; rate constants given in units of \( 10^{-10} \text{ cm}^3 \text{ s}^{-1} \).

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Ionic Products</th>
<th>( k \ 300 \text{ K} )</th>
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<tr>
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<tr>
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<tr>
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INTRODUCTION:

Intermolecular H/D exchange experiments in mass spectrometers have typically been done in the ion source using chemical ionization (CI) and more recently using D₂. The application of tandem mass spectrometry to H/D exchange offers several advantages, including the ability to study specific compounds in complex mixtures and the ability to control the kinetic energy of the ion and the number of collisions it undergoes.

Beam induced H/D exchange using D₂ is a useful method for the analysis of non-volatile and thermally labile organic compounds. Because ND₃CI is a transparent matrix, it does not contribute any ions to SIMS spectra in contrast to the behavior of many liquid matrices. This work describes the analytical potential of two types of exchange experiments, both with respect to determining the number and types of active hydrogens and their implications for the mechanisms of I/M reactions.

EXPERIMENTAL:

A Finnigan model 4500 triple stage quadrupole (QQQ) and a custom-built pentaquadrupole (QQQQQ) mass spectrometer were used to carry out exchange experiments in the collision region. Mass-selected parent ions (either M⁺ generated by 70 eV EI or M+H⁺ generated by 70 eV CI) were allowed to collide with a deuterated reagent gas (pressure 2 mTorr) in the rf-only quadrupole at a collision energy of 1.5 eV.

D₂ experiments were performed using a Riber SIMS. Ar Ion beam of 4.5 keV bombarded a silver surface covered with a mixture of analyte and ND₃CI.

RESULTS AND DISCUSSION:

H/D Exchange in the Collision Region

The degree of D incorporation is dependent on the proton affinity difference between the analyte ion and the reagent (ΔPA) and also on the kinetic energy of the analyte ion. Reagents can be chosen which exchange all active hydrogens or only selective hydrogens. For example, CH₃OD (PA 181.9 kcal/mol) exchanges only with the carboxylic group of the radical ion of anthranilic acid (PA of benzolic acid 198.2 kcal/mol; of aniline 209.5). ND₃ (PA 204.0) exchanges both the amino and carboxylic hydrogens. The selective exchange of carboxylic hydrogen in the presence of amino hydrogens by CH₃OD was confirmed by pentaquadrupole experiments (Fig. 1). A reaction mechanism (Scheme 1) has been proposed to explain the experimental observations.

An investigation of the effects of collision energy reveals that (I) cluster ion formation (II) the H/D exchange process and (III) low energy collision activated dissociation due to reactive collisions are all intimately connected as shown in Fig. 2. Note that at very low energy (1-2 eV) argon does not cause any fragmentation to protonated ion of benzoic acid (Fig. 2A) whereas CH₃OH does (Fig. 2B). The % D incorporation (Fig. 2C) and the signals due to methanol clusters, I/M complexes and to the fragment C₇H₅O⁺ (m/z 105) decrease as the collision energy is increased. Although the formation of the I/M complex is initially driven by ion-dipole and Ion-induced dipole interactions, the exchange process is far more complicated and cluster ion formation plays a major role.

H/D Exchange during DI

The degree of exchange in the desorbed ions provides insights into the mechanism of interfacial chemical reactions occurring in the selvedge region during DI. For example, the intact cation (m/z 162) of carnitine HCl shows 2 active hydrogens whereas its trans-alkylated product (m/z 176) shows only one active hydrogens (Fig. 3) which is in agreement with the proposed mechanism.

The relative abundance of ions resulting from exchange is dependent upon the primary argon ion dose (Fig. 4). These data suggest that the exchange is induced by ion beam and does not occur in the matrix prior to ion bombardment.

CONCLUSIONS:

Both methods are valuable in counting the total number of active hydrogens and in obtaining mechanistic information on I/M reactions. Exchange in the collision region shows that counting certain types of active hydrogens in polyfunctional compounds is possible and cluster formation and endothermic reactive collision play an important role in the mechanism of H/D Exchange. Exchange in SIMS using ND₃CI shows that the exchange process is beam induced and hence this is a valuable tool in studying interfacial chemical reactions.

REFERENCES:

**FIGURE 1:**

**SCHEME 1:**

**FIGURE 2:**

**FIGURE 3:**

Carnitine HCl + NH₄Cl + Ag

**FIGURE 4:**

Adamantamine HCl + ND₄Cl + Ag
Substituent effects, steric effects, and both conformational and configurational effects play important roles in the outcome of organic reactions in both the solution and gas phase. We have undertaken a study of the effects of functional group interactions on the reactive and dissociative behavior of a series of alkenols and amino-alcohols using an ion trap mass spectrometer. In solution, hydroxy and amino groups may act as hydrogen bond donors and hydrogen-bond acceptors, and double bonds may act as hydrogen-bond acceptors, so the influence of these functional groups was of primary interest in gas-phase studies. The objectives were to examine the ion/molecule reactions of dimethyl ether ions with a series of aminoalcohols with varying functional group separation, to probe hydrogen-bonding effects in alkenol adduct ions, and to characterize the product ions of these ion-molecule reactions by using low energy collisional activation.

It has been proposed that the pπ electrons of a double bond may coordinate a proton attached to another site, so we undertook a study of the effects of this potential functional group interaction on the reactive and dissociative behavior of simple bifunctional molecules. We have examined the collisionally activated dissociation (CAD) spectra of the ion/molecule reaction products of a series of alkenols (allyl alcohol, 3-buten-1-ol, 4-penten-1-ol) to correlate functional group separation and hydrogen-bonding interactions.

Ion/molecule reactions of the alkenols with dimethyl ether result in two adduct ions of interest: (M + H + DME)+ and (M + H + DME - H2O)+. A mechanism proposed for the formation of (M + H + DME - H2O)+ from (M + H + DME)+ (Scheme 1) involves an ion-molecule complex as an intermediate step. The abundance of the dehydration product, (M + H + DME - H2O)+, relative to (M + H + DME)+ increases as the length of the carbon chain decreases. The ratio of (M + H + DME - H2O)+ to (M + H + DME)+ is 1:1 for allyl alcohol, 1:2 for 3-buten-1-ol, and 1:4 for 4-penten-1-ol. The heats of formation of each alkenol/DME complex were estimated and varied by as little as 3 kcal/mole, so it is unlikely that the exothermicity of the reaction alone can account for the large differences in stability of the adduct.

This suggests that intramolecular interactions between the double bond and the proton provide additional stabilization for the proton-bound complex relative to the dehydrated complex. The increased length of the carbon chain in 3-buten-1-ol and 4-penten-1-ol allows more favorable intramolecular interactions between the double bond and the proton because stable six and seven membered ring structures are formed. Likewise, the formation of a proton bridge within the allyl alcohol is presumably less favorable due to the smaller size of the resulting ring structure (a five-membered ring). For the (allyl alcohol + H + DME)+ adduct, however, the relative strengths of the hydrogen bonds between the allyl and DME portions are more equivalent, and this may enhance the lifetime of the proton-bound complex, allowing more time for the rearrangement resulting in (M + H + DME - H2O)+ (shown in Scheme 1B) to occur.

As a comparison, simple alcohol adduct ions were also examined to probe the importance of the double bond functional group on the formation and dissociative behavior of the adducts. Abundant protonated ions and proton-bound dimers were observed, although the formation of (M + H + DME - H2O)+ only occurred to a minor extent. This latter observation likely reflects the lack of favorable intramolecular hydrogen-bonding which assisted the rearrangement shown in Scheme 1, route B. Such interactions play an important role in stabilizing the ion/molecule complex and in enhancing entropically the extensive rearrangement needed for elimination of H2O.

Thus, the formation and dissociation of the alkenol/dimethyl ether and alcohol/dimethyl ether adducts is dependent on the propensity for formation of intramolecular hydrogen bonds within the adducts. For substrates with functional group separations that enhance formation of stabilizing intramolecular hydrogen bonds (such as within 4-penten-1-1-ol), adducts may preferentially dissociate via different routes than the adducts of those substrates (i.e. allyl alcohol) for which the intermolecular hydrogen bonds between the two molecules of the complex are more thermochromically equivalent.

We have also undertaken a study of the reactive and dissociative behavior of a series of amino alcohols and related molecules. In order to evaluate the significance of functional group interactions in gas-phase ion chemistry, compounds differing both in the separation of functional
groups and in their substitution patterns were chosen. The adduct ions of interest (M+13)+ were formed via ion-molecule reactions between neutral amino alcohols (M) and dimethyl ether ions (CH₃OCH₂)+ in a quadrupole ion trap mass spectrometer. The adduct ions were then characterized by CAD. Two mechanisms are proposed for the formation of these adducts. Both involve nucleophilic attack of the amino alcohol on the dimethyl ether cation, followed by elimination of methanol to give net addition of a CH group. The only difference between these two mechanisms is the reactive site (hydroxyl or amino group) of the amino alcohol.

To elucidate the justification for these competitive mechanisms, ethanolamine, N-methylethanolamine, and N,N-dimethylethanolamine were studied. All formed (M+13)+ adducts upon reaction with dimethyl ether ions. The CAD spectra for these adducts (Figure 1) show that both mechanisms may be operative. In the case of (ethanolamine + 13)+, the major fragment corresponds to loss of water. On the other hand, the spectrum for the tertiary amino compound shows only loss of 30 daltons (loss of formaldehyde). N-Methylethanolamine represents the intermediate case, in which both of these losses are observed in significant amounts.

The effect of functional group separation on the dissociative behavior of amino alcohol adducts is most dramatically illustrated by the differences in the CAD spectra of 3-amino-1-propanol and 2-amino-1-propanol (Figure 2). Whereas the CAD spectra for the simple protonated molecules are very similar, showing predominantly loss of water, the (M+13)+ adducts show strikingly different behavior. The 2-amino-1-propanol (M+13)+ adduct gives primarily loss of water and loss of formaldehyde. On the other hand, the 3-amino-1-propanol adduct gives only 44+ ions and a very slight amount of formaldehyde loss.

This investigation has shown that the effect of interfunctional distance on the reactive and dissociative behaviors of amino alcohols can be dramatic. Also, while the degree of substitution on the amine functionality is important in determining adduct formation and fragmentation patterns, the substitution of the carbon chain connecting the amine and hydroxyl functions seems to have little effect on the reactivity of the system.

This work was supported by grants from the Welch Foundation, the Petroleum Research Foundation, and an ASMS Young Investigator Research Award.

![Figure 1](image1)

![Figure 2](image2)

Scheme 1. CAD of Proton-Bound Dimers
Ionized Cycloalkane Formation from Ionized n-Butanol

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Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550 and Department of Marine Sciences, Texas A&M University at Galveston, Galveston, Texas 77553

Ionized cyclopropanes are common products of radical cation fragmentations in the gas phase [1]. Most of these reactions take place by the cyclization of distonic intermediates (Scheme 1) in which R is H or an alkyl group. Y can be OH, OR, NH2, etc., and n > 1.

Scheme 1

\[
\begin{align*}
RCH_2Y^+ & \rightarrow RCHY^+ \rightarrow RCH^+ + YH \\
\end{align*}
\]

Since the formation of ionized cyclopropane [1] and methylcyclopentane [2] is well established, a search for ionized cyclobutane was needed to define further the generality of Scheme 1. The objective of this investigation was to characterize C4H9+ product ions formed by water elimination from ionized n-butanol to define better the scope of ionized cycloalkane formation in gas phase ion chemistry. Formation of ionized methylcyclopropane from this ion has recently been proposed [3].

All experiments were performed on an Extrel FTMS-1000 equipped with a 3 T superconducting magnet and 5.08 cm cubical cell. Spectra were collected in the direct mode under broad band conditions. Ions were excited by a 40 Vpp chirp from 0 to 3.66 MHz at 2 KHz/us. Daughter ions were produced from butanols by electron impact at 12 eV. The trapping voltage was 2 V. The typical pressures for butanols and NH3 were 6-8x10^{-8} and 2-3x10^{-7} torr respectively.

We used reaction with NH3 to characterize C4H9+ fragments, as 1 and 2 react with NH3 to give characteristic products (Scheme 2). Formation of C4H9+ upon reaction with NH3 distinguishes 2 from 1.

Scheme 2

\[
\begin{align*}
\text{CH}_3\text{CH}^+ \rightarrow \text{CH}_2^+ \quad + \quad \text{NH}_3 \\
\end{align*}
\]

Products formed by reaction of C4H9+ from 1-4,4,4-d and from 1-3,3-3-d with NH3 are given in Table 1. C4H9D+ from 1-3,3-3-d, the daughter ion expected following 5-membered ring H-transfer, gave products at m/z 30, 31 and 45 upon reaction with NH3. The m/z 45 demonstrates formation of 1. This is confirmed by the production of C4H9NH2+, C4H9D,N2+ and
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C,H,D,N in the reactions of C,H,D, from 1-4,4,4-d with NH,. This reaction also produced m/z 30 (CH NH +), m/z 31 (CH NH or CH N'), m/z 32 (CH ND or CH,NH'). Our observations suggest formation of 1 as follows (Scheme 3):

Scheme 3

Considerable H shuffling is apparent in the products of the reaction of NH with C'H,D, formed from 1-4,4,4-d (Table 1). This is explained by H-exchange between the partners in an encounter complex rather than in "CH(CHCD),NH", since exchange does not occur between N and S-carbons in ionized amines. C,H,D, ions formed by 6-membered ring H-transfer from 1-3,3-d and 1-4,4,4-d formed substantial one carbon but no two carbon products upon reaction with ammonia (Table 1). The elimination of water from ionized 1-butanol produces 2 following 6-membered ring H-transfer and 1 following 5-membered ring H-transfer. The C,H,D:,C,H,D, ratio from 1-3,3-d (3.3) and C,H,D:,C,H,D, ratio from 1-4,4,4-d (3.2) indicate that 2 was produced from ionized n-butanol more than 3 times as often as 1 at 12 eV electron energy.

The formation of ionized cyclobutane and methylcyclopropane establishes that isomerization to a distonic intermediate followed by cyclization with elimination of a stable neutral fragment (Scheme 1) is a common mass spectral fragmentation. Ionized cyclobutane is formed from ionized 1-butanol more than 3 times as often as is ionized methylcyclopropane. The distribution of products in these reactions is largely determined by the relative rates of the H-transfers that form the distonic intermediates.


Table 1. Products of Reactions of C,H,D, with NH,

<table>
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<th>CH,CD,CH,OH</th>
<th>CD,CH,CH,OH</th>
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<tr>
<td>C,H,D,</td>
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<td>100</td>
</tr>
<tr>
<td>C,H,D,</td>
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</tr>
<tr>
<td>C,H,D,</td>
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</table>

Results were obtained at 12 eV ionizing electron energy. Product distributions were determined as described in the text.
THE MECHANISM OF THE GAS-PHASE BASE CATALYZED ALDOL REACTION: REACTIONS OF SUBSTITUTED BENZALDEHYDES WITH ACETONE ENOLATE ANION.

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Base-catalyzed aldol condensations have an important role in modern organic synthesis. Yet the gas-phase aldol condensations are not well-understood. Consequently, the goals of this study are (1) to establish a gas-phase analogy for the aldol condensation, (2) to investigate the extent of the tetrahedral complex versus loosely-bound complex (i.e., ion-dipole and proton-bound species), and (3) to look for substituent effects.

In two previous studies of the reaction of the acetone enolate and neutral acetone, it was found that the gas-phase adduct possesses three structures:

1. Tetrahedral in which there is a covalent bond between the nucleophile and the acetone.
2. Proton-bound in which two enolates are held together by a proton.
3. Ion-dipole in which the enolate and acetone are bound by electrostatic forces.

Structures (2) and (3) are regarded as "loosely-bound".

In this study, we have used the reaction of acetone enolate anion with a number of para-substituted benzaldehydes to probe the extent of tetrahedral adduct formation as a function of Hammett $\sigma$ substituent parameters. Accordingly, the system allows us to investigate the extent of the negative charge by various substituents in the formation of tetrahedral adduct. Furthermore, we can examine quantitatively the correlation of tetrahedral adduct formation and substituent effects by using the Hammett equation. Thus, the experiment can be viewed in both a qualitative and quantitative manner.

The data show that the losses of water from the adducts and from the reference compounds increase as the electron withdrawing character of the substituents increases. The CAD spectra of adducts from the most electron-withdrawing and the most electron releasing groups studied are shown in figures 1 and 2. The increasing propensity for the adduct to lose water is interpreted to indicate increasing extent of tetrahedral adduct formation. In this adduct, the negative charge is stabilized when the substituent is electron withdrawing.

As mentioned earlier, to test quantitatively the correlation of the extent of tetrahedral adduct formation with substituent effects, a Hammett plot was prepared (see figure 3). Figure 3 displays all the para-substituents used and has a correlation of $+1.48$, and a p-value (slope) of $+1.49$. The reaction constant ($p$) indicates the reaction is facilitated by electron-withdrawing groups and is moderately sensitive to substituent effects.

Thus from the data obtained, a couple of conclusions can be drawn:

1. The extent of tetrahedral adduct formation is greatest for systems in which an electron withdrawing group is introduced.
2. The Hammett plot shows that the reaction constant is positive, giving a quantitative measure of how the formation of tetrahedral adducts are facilitated by electron withdrawing groups.

REFERENCES

Figure 1. A) CAD spectrum of the adduct of m/z 208 formed in the gas-phase reaction of the acetone enolate and 4-Nitrobenzaldehyde. B) CAD spectrum of the reference aldol product of m/z 208 formed in the condensed-phase reaction of the same reactants.

Figure 2. A) CAD spectrum of the adduct of m/z 206 formed in the gas-phase reaction of the acetone enolate and 4-(Dimethylamino)-benzaldehyde. B) CAD spectrum of the reference aldol product of m/z 206 formed in the condensed-phase reaction of the same reactants.

Figure 3. TETRAHEDRAL GEOMETRY vs LOOSELY-BOUND GEOMETRY
HAMMETT CORRELATION

<table>
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<th>SIGMA</th>
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<th>H3C-N-(CH3)2</th>
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<td>1.5</td>
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GRAPH WITH THID SUBSTITUENT
CORRELATION = 0.67
Rho = 1.48
Mass spectrometry played a key role in environmental research and environmental analytical chemistry during the 1980s. One major reason for the significant growth in applications of mass spectrometry in environmental studies was the development of analytical methods that were incorporated by the United States Environmental Protection Agency (USEPA) as approved methods into regulatory programs under the Safe Drinking Water Act, the Clean Water Act, and other environmental legislation. These approved analytical methods consisted of detailed cookbook-style descriptions of all the individual method steps from sample collection to production of qualitative and quantitative results. The methods were incorporated into the Federal regulations to ensure uniform and comparable analytical results from mandated environmental analyses.

During the 1980s three general USEPA mass spectrometric analytical methods came into widespread use, and slightly different versions of each evolved to meet the perceived different requirements of similar, but legally different regulatory programs:

- **Methods 624, 524.1, 524.2, 8240, 8260, and CLP/VOC.** Volatile organic compounds (VOCs) by inert gas purge, trapping of the vapors on a solid sorbent, and thermal desorption into a packed or capillary column gas chromatography/mass spectrometry (GC/MS) system.

- **Methods 625, 8250, 8270, and CLP/SEMIVOL.** Gas chromatographable compounds by methylene chloride (CH₂Cl₂) extraction, phase separation, concentration of the CH₂Cl₂ extract by evaporation, and injection of an aliquot into a packed or capillary column GC/MS system.

- **Methods 613, 513, 8290.** 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and some congeners (8290) by solvent extraction, phase separation, concentration of the solvent extract by evaporation, and injection of an aliquot into a capillary column GC/high resolution MS system operating in the selected ion monitoring mode at about 7500 resolution.

Although the regulated community had the choice (except for dioxin) of an approved mass spectrometric or an approved non-mass spectrometric method, the mass spectrometric methods became very popular because of their power and cost-effectiveness. The demand for mass spectrometric analyses provided a great boost to instrument sales which led to many improvements in commercial instrumentation.
During the 1990s, applications of mass spectrometry in environmental research and analyses should continue to grow. Some predictions of future directions in research and environmental applications of mass spectrometry are:

- The multiple but similar mass spectrometric methods, which developed during a period of changing technology (packed to capillary GC columns) and perceived differences in regulatory programs, will undergo consolidation and restructuring to fewer and more uniform methods across legally different but similar regulatory programs.

- Lower regulatory limits for metals and toxic elements in environmental samples will lead to further development and widespread application of mass spectrometric methods for these substances, e.g., inductively coupled plasma (ICP)/MS.

- Concern for environmental contamination by non-gas chromatographable (nonvolatile) organic and inorganic compounds will lead to extensive development and regulatory application of liquid chromatography (LC)/MS methods. An early indication is the recently developed USEPA Method 553 for the determination of benzidines and nitrogen-containing pesticides in water by liquid-liquid extraction or liquid-solid extraction and reverse phase high performance LC/particle beam/MS.

- Concern for water soluble and non-extractable organic and inorganic environmental contaminants will lead to new analytical methods based on semi-permeable membrane sampling systems and membrane mass spectrometry (M/MS).

- Process analytical chemistry will incorporate mass spectrometric methods, especially M/MS and bench-top tandem MS, for continuous monitoring of process and waste streams to feed back real-time analytical results to maintain process control and prevent pollution at the source.

- Concern for human and ecological exposure assessment will provide considerable research opportunities to apply emerging mass spectrometric methods to the study the interactions of biomolecules and environmental contaminants.

- Electron capture negative ionization mass spectrometry will become more widely used for environmental analyses. The advantages of high sensitivity and high selectivity for compounds with electronegative elements or substituents will be required and the problems of weak standardization among different laboratories and a lack of standard reference data will be solved.

Overshadowing all this promise is the question: where will all the analytical chemists and mass spectroscopists come from? Unless sufficient well-trained analytical chemists with mass spectrometry skills are produced, some or all of these and other promising areas will not develop.
There exists an analytical chemistry equivalent of the Heisenberger uncertainty principle of physics. As the concentration of the analyte being measured falls, the uncertainty of the result increases rapidly. In the 1980’s mass spectrometry displayed the quantum jump to routinely measure levels as low as parts per trillion (ppt) brought about mainly by such environmental issues as dioxins and polychlorinated biphenyls. Additionally the risk assessments pronounced by toxicologists have often placed ‘safe levels’ in the low ppb levels. The quest to determine whether a substance is present or not revolves around the ability to detect down to the ultimate level of one molecule - the search for zero. For the moment, however, the measurement of trace levels to the ppt level represents the lower limit of detection by mass spectrometry.

Scientists are not exempt from the mundane rigors of supporting their findings. In a court of law it is required to provide proof in a criminal case that is “beyond reasonable doubt” or in a civil matter that “the preponderance of evidence” supports the conclusion. Since methods of confirmation by mass spectrometric techniques are often developed on an ad hoc basis to deal with an emergency situation, they cannot be validated by interlaboratory testing. What has emerged over the last decade, however, is the evolution of a set of criteria generally recognized as scientifically sound (1).

HISTORICAL BACKGROUND

The highest level of confirmation that can be provided by mass spectrometry is the exact correlation between the full mass spectral scans of a reference standard and the sample performed within the same working day. Usually El spectra contain sufficient structurally related fragment ions to permit absolute identification. Under such conditions, the relative abundance ratios should experimentally fall within 5%. Quite often, however, the presence of background ions may severely interfere with exact comparisons. This practice of direct comparisons represents the highest level of specificity obtainable by mass spectrometry.

In the field of trace level analysis, however, the opportunity to employ full mass spectral scans as a means to confirmation of presence is often an impractical situation. To make full use of sensitivity of detection, the technique of multiple ion monitoring (MID) offers a convenient method of ignoring potential interferences and concentrating on ions belonging to the compound under investigation. It is in this particular sphere of activity that the evolving criteria for confirmation have received greatest deal of attention. Scrutiny has been focused on the exact number of ions to be monitored to provide proof of presence. This examination of criteria for confirmation has been complicated because of the myriad of mass spectrometric techniques available for analyzing food samples. It was argued more than a decade ago that a minimum of three structurally related ions would be necessary to provide proof of presence (2). To improve the criteria for confirmation the relative abundance ratios were required to be within 5% when compared with a reference standard recorded under similar conditions. Evolution of new techniques, particularly soft ionization methods, has prompted a re-investigation of supporting evidence for confirmation since little or no fragmentation is observed.

CRITERIA FOR CONFIRMATION

1. Full Mass Scans

In the case of full mass spectral scans derived under the various ionization techniques (El, CI, NCI, FAB, etc.), the reference standard and the sample recorded under similar conditions on the same instrument should agree with no less than a 5% agreement in relative abundance ratios. This logic, while obvious, is often difficult to practice experimentally. While reference standards are pure compounds, the sample extract can introduce interfering ions into the mass spectrum complicating the confirmation process. Using chromatographic separation prior to mass spectrometry can often guarantee that interfering ions are reduced to a minimum allowing direct spectral matching to take place. However, when probe samples are used for confirmation, then gross interference can occur. In the case of confirmation of aflatoxin B1 in peanuts under NCI, Park et al. (3) argued that although the three ions representing the compound (m/z 297, 311, and 312) were present in the correct relative abundance ratios, they could be fragment ions from higher molecular weight compounds. These authors have stated that when the interfering ions constitute greater than 20% of the total intensity of the mass spectrum, then confirmation cannot be deduced in spite of the three ions at the correct relative abundance ratios. This lower limit of 20% represented a responsible judgment by the authors based on practical experience, i.e. the expert opinion factor.
2. Multiple Ion Detection

Confirmation of trace levels are generally carried out using multiple ion detection techniques to lower the detection limit of the mass spectrometer to match the residue problem. This method of confirmation mandates that chromatographic separation of the sample be performed prior to mass spectral analysis to concentrate the compound of interest into an appropriate elution profile for analysis as well as potential quantification if desired. The literature contains a large number of documented case histories where more than three or four ions were adopted for the confirmatory process. For example, in the case of trace levels of dimethoate in mangoes (4), four ions under GC/MS using methane CI were selected for confirmation of the eluting peak believed to represent the pesticide in the extract. It would seem that the prevailing expert consensus prefers to adopt no less than four ions for confirmation. These case histories can therefore be considered to establish that a minimum of three structurally related ions are generally recognized as scientifically sound. In the case of quantification, however, a single ion is often used to further reduce the limit of detection, optimize dwell times and improve precision and accuracy. This method of approaching quantification is generally accepted only after confirmation has been performed or a deliberate study is underway using spiked samples in a recovery study.

3. Product Ion Chemistry

Soft ionization methods have become popular techniques for the analysis of trace level residues because they provide two distinct advantages over EI. First, the ionization process favors the production of solitary protonated molecule ions or adduct ions depending on the reagent gas employed. Second, soft ionization methods tend to suppress the background interference ions due to lack of fragmentation. Observation of protonated molecule ions can be considered the most important criterion for identification, but the burden of proof of presence placed on a single ion species cannot be regarded as sufficient for confirmation. The majority of residue samples analyzed by liquid chromatography/mass spectrometry (LC/MS) suffer from this same disadvantage that chemical ionization methods provide only protonated molecule ions for confirmation. Since molecules analyzed by LC/MS are usually thermally labile or non-volatile, the opportunity to use EI to obtain sufficient fragment ions is not an experimental option. Only two LC interfaces have the option to perform EI studies, the moving belt interface and the particle beam device. Therefore, the degree of specificity has been increased by using tandem mass spectrometry (MS/MS). In the case of etrimphos, both the EI and CI spectra did not contain sufficient fragment ions for the confirmation process. However, the product ion spectrum derived from the protonated molecule ion, m/z 292, did provide five ions to meet the confirmation criteria (5). Using the protonated molecule ion as the precursor ion, the product ion spectra usually contain sufficient ions to meet the criteria of a minimum of three structurally related product ions. Therefore, the recent shift in emphasis to the use of LC/MS interfaces has created a reliance on product ions spectra to satisfy the criteria for confirmation.

CONCLUSIONS

Although the variation in the application of MS methods to residue analysis is large, the underlying criterion that at least three structurally significant ions are necessary for confirmation has been experimentally established to be valid. Most members of the discipline, however, have elected to go beyond the minimum criteria whenever possible. With the increasing application of soft ionization methods established by the various LC/MS methods, the lack of specificity has been overcome by the use of LC/MS/MS. The use of product ion spectra derived from protonated molecule ions has become a practical solution to analyzing many thermally labile compounds. Most of the problems in analyzing food samples involve relatively low molecular weight compounds. The choice of criteria for confirmation outlined above have been selected with this molecular weight range in mind. However, the next generation of pesticides derived by biotechnology will involve much higher molecular weight compounds. With increasing molecular weight the criteria for confirmation must be expected to undergo a revision to increase the number of ions required. However, the increased use of collision experiments via MS/MS will be expected to eventually replace the conventional approaches to confirmation through product ions derived from molecule ions.

REFERENCES

The Impact of Electrospray on Environmental Monitoring

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Introduction

Environmental monitoring of many non-volatile or thermally unstable polar organics by LC/MS relies on the development of sensitive, specific and cost effective LC/MS techniques. Electrospray can meet these goals since it has the capability to generate molecular ions (i.e. [M+H]⁺ or [M+Na]⁺) from low pg quantities of many environmentally relevant compounds. Fragment ions for identification or confirmation can be generated by multiple mass analyzer instruments, but more cost-effective approaches such as collisional activation in the electrospray transport region or the use of ion trap mass spectrometry (ITMS) can provide this structural information. Collisional activation decomposition (CAD) in the transport region of the electrospray interface or in an ITMS can result in higher daughter ion yield than typically achieved from triple quadrupole MS. This paper reports on electrospray combined with MS for the determination of numerous small molecular weight compounds of environmental interest.

Experimental

An electrospray source (Analytical of Bradford) was interfaced to a Finnigan MAT 4500 quadrupole MS. Samples ranging from 1 pg to 100 ng/µL were introduced into the RS source at a flow rate of 2 µL/min using a syringe pump. The potential difference across the capillary and skimmer in the ion transport region of the FS source could be used to control the extent of fragmentation. An electrospray Interface (Vestec) was also mounted to an ITMS (Finnigan MAT) to analyze several of the same samples under analogous operating conditions.

Results and Discussion

The main goal in this study was to evaluate electrospray MS for generating of structurally relevant information on typical molecules of environmental interest. One compound included in this study was aldicarb. Aldicarb, a thermally labile carbamate pesticide, exhibits fragmentation (ion at m/z 116) for a very low capillary/skimmer potential difference of 10 V (Figure 1). At 50 V potential difference the [M+Na]⁺ and other adduct ions are dissociated and the fragment ion at m/z 89 and m/z 116 increase in intensity. At a higher potential difference of 85 V only low mass fragment ions such as CH₃Na⁺, C₂H₅⁺ and C₃H₇⁺ are formed. Other pesticides and herbicides investigated also exhibit significant structural fragmentation at various capillary-skimmer potential differences. In general, potential differences in the 0-20 V range do not exceed the activation energy for the lowest energy fragmentation process, but remove most cluster ions formed through hydrogen bonding. In the 60-100 V potential differences range significant breakage of covalent bonds occurs while the signal for the molecular ion is retained. Potential differences above 100 V usually result in low mass fragments. At a high potential difference (140 V) over 16 eV of internal energy can be imparted into the molecule.

Besides providing structural information, the sensitivity of electrospray for detecting environmentally significant compounds proved to be excellent. Transmission of ions throughout the transport region at various capillary-skimmer potential differences varied by less than 20%. This means that the sum of the daughter ion currents at a high capillary-skimmer potential difference (CAD conditions) was nearly identical to the [M+H]⁺ ion current for a low capillary-skimmer voltages (non-CAD). The efficient CAD process permits detection of daughter ion for the analysis of 10 pg of aldicarb when scanning from 10-500 daltons (Figure 2). The sensitivity and linearity for electrospray MS can be seen in Figure 3 for a calibration curve of aldicarb from 10 pg - 100 ng. Concentrations above 100 ng often result in saturation of the electrospray ionization process causing a drop or leveling in the MS response.

In order to achieve better sensitivity, the combination of electrospray with an ion trap mass spectrometer (ITMS) was evaluated. Preliminary data generated from the coupling of the techniques indicated slightly better detection limits could be achieved for aldicarb. However, we are still evaluating and optimizing the electrospray - ITMS interface and anticipate significant improvements in sensitivity compared to quadrupole MS.

Acknowledgements

This work was supported by EPA Contract No. 8-02-4544.
Figure 1. ES determination of 2 ng of aldicarb in 1:1 MeOH:H₂O at a flow rate of 2 μL/min.
A) Spectrum of aldicarb at capillary-skimmer potential difference of 10 V
B) Spectrum of aldicarb at capillary-skimmer potential difference of 50 V
C) Spectrum of aldicarb at capillary-skimmer potential difference of 85 V

Figure 2. CAD spectrum of 10 pg of aldicarb at a capillary-skimmer potential difference of 50 V. (1:1 MeOH:H₂O at a flow rate of 2 μL/min.)

Figure 3. Electrospray MS calibration curve for aldicarb.
Applications of Mass Spectrometry to the Analysis of
3-Chloro-4-(Dichloromethyl)-5-Hydroxy-2(5H)-Furanone (MX).

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Introduction. Existing methods for the analysis of the potent mutagen 3-chloro-4-chloromethyl)-5-hydroxy-2(5H)-furanone (MX) are unsuitable because they either rely on external standardization or internal standardization. Improved accuracy, particularly when using mass spectrometry can be obtained by internal standardization because any changes in the response of the analyte, due to the presence of other compounds or changes in ion source conditions are normalized to the response of the internal standard. The internal standardization method is however a semi-quantitative method because the response of MX to the internal standard is determined by measuring the response of a single concentration of MX to a single concentration of the internal standard. The objective of this study was therefore to develop an internal standardization method for the analysis of MX in waters. Accordingly, the mass spectrum of the methyl derivative of MX was characterized, the need for high-resolution mass spectrometry was established and a method was validated and tested that uses isotopically labelled benzoic acid as an internal standard.

Experimental. Solutions of the methyl derivative of MX and the methyl ester of benzoic acid were prepared by reacting standard materials of MX with solutions of 2% H₂SO₄ in methanol, deuterated methanol for 1 hour or 14% (v/v) boron trifluoride (BF₃) in methanol for 12 hours in a 70°C mineral oil bath. The sulfuric acid and boron trifluoride was neutralized by the addition of 2% aqueous NaHCO₃ and the methylated products were extracted into hexane.

Experiments were performed on a Hewlett-Packard 5890 gas chromatograph interfaced to a VG70-250SEQ mass spectrometer operating at a resolving powers of 1,000, 10,000 or 20,000 (10% valley definition). A 30m, 0.32mm I.D., 0.25um film, DB-5 fused-silica capillary column was used in all experiments. The GC oven was held at an initial temperature of 115°C for 3 min. and then increased to 165°C at a rate of 6°C/min in experiments performed to characterize the mass spectrum of the methyl derivative of MX. In other experiments, the GC oven was held at an initial temperature of 50°C for 1 min. and then increased to a temperature of 170°C at a rate of 10°C/min. Mass spectra were acquired by using electron ionization at an electron energy of 70 eV, 200 uamp trap current, source temperature of 150°C and interface temperature of 250°C. Ions corresponding to the molecular ion of the methyl ester ¹³C₆ benzoic acid (m/z 142.0726) and a PFK lock mass ion m/z 142.9920 were monitored from 3-11 minutes and fragment ions of the methyl derivative of MX (m/z 198.9121, 200.9024) and a PFK lock mass ion m/z 192.988 were monitored from 11-13 minutes in analyses performed by selected-ion-monitoring. Mass spectrometry/mass spectrometry experiments were conducted by introducing standard material of the methyl derivative of MX through the gas chromatograph and acquiring the MS/MS spectrum during the elution of the MX peak. The ion at m/z 229 was transmitted from MSI at a resolving power about 500 to the collision region, where collisionally activated decompositions (CAD) to product ions occurred. Conditions in the collision-region were an indicated argon pressure on the Bayert-Alpert ion gauge of 5x10⁻⁵ mbar and a collision energy of 25 eV.

Results and Discussion. Previous interpretations of the electron-ionization mass spectrum of the methyl derivative of MX has suggested that the isotope cluster beginning at m/z 199 is due solely to the (M-OCH₃) fragment ion. Measured abundances of the isotope ratios at m/z 199, 201 and 203, however differ from the theoretical values for a three chlorine isotope cluster (Figure 1). We hypothesized that the cluster arises from two distinct pathways and is composed of two fragment ions, each containing three chlorine atoms. One fragment arises from the loss of the -OCH₃ from the molecular ion. The other is due to either the loss of -OCH from the molecular ion or -CO from the (M-1)⁺ ion to yield a fragment ion at m/z 201 that overlaps with the three chlorine cluster at m/z 199. Experiments were designed to test this hypothesis. Accordingly, we deconvoluted the original cluster at m/z 199 assuming the presence of a three chlorine isotope cluster at m/z 199 and another at m/z 201 with good agreement between calculated and theoretical relative abundances of ions. The
presence of these two fragment ions was confirmed by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) at a resolving power of 20,000. The mass spectrum of MX, methylated with deuterated methanol showed two fragment ions, one which arose from the loss of \(-\text{OCD}_3\) (m/z 199) and the other from the loss of \(-\text{OCH}\) (m/z 204). Mass spectrometry/mass spectrometry (MS/MS) of the m/z 229 (M-1\(^+\)) fragment ion of the methyl derivative of MX showed a product ion at m/z 201. The fragment ion at m/z 201 therefore originates from the loss of \(-\text{CO}\) from the (M-1\(^+\)) ion.

Selected-ion-monitoring of the ions at m/z 199 and 201 is recommended in the analysis of MX and thus the use of high-resolution mass spectrometry was explored because we do not know the effect of instrumental parameters (e.g., ion source temperature) on the relative yields of the two fragment ions, and thus on the observed ratio between the ions at m/z 199:201. We do know however that at a resolution about 10,000 the ions at m/z 201 are resolved. Operating under high-resolution conditions then allows the use of confirming criteria based on the theoretical ratio of ions in a three chlorine isotopic cluster. Doubt about the presence of the compound, in situations where the compound is present near the detection limits of the method, can thus be reduced because one is comparing an experimental (variable) value to a known (fixed) value instead of two experimental values that are by definition, variable. Additional need for high-resolution mass spectrometry was demonstrated by comparing the results of analyses of a chloramination extract performed at a resolving power = 1,000 and 10,000. Under low-resolution conditions, identification and quantification of MX was impossible due to low signal:noise values. In this case, noise due to chemical interferences was eliminated by using high-resolution mass spectrometry resulted in an improvement in the S:N value of an order of magnitude. A high-resolution mass spectrometry method for the analysis of MX was therefore developed that uses isotopically labelled benzoic acid as an internal standard. Validation of the method was achieved by spiking a known amount of MX into lake water and into chlorinated lake water. The difference between the expected and measured values was less than 10%. The response of the methyl derivative of MX to the methyl ester of \(^{13}\text{C}_6\) benzoic acid was shown to be linear from 50 pg/ul to 1000 pg/ul. A deviation from linearity was observed at lower levels (10 pg/ul) and the method was shown to have a detection limit (S:N = 3:1) of about 20 pg/ul. Thus, the method is applicable to the analysis of MX in a broad range of waters.

Figure 1. Mass spectrum of the methyl derivative of MX. The fragment ion at m/z 199 has been attributed to the loss of \(-\text{OCH}_3\) from the molecular ion. In this case, the chlorine isotope cluster would be similar to that observed in the m/z 229 fragment ion.
CHEMICAL TRANSFORMATION BROADENS THE SCOPE OF MS: DNA Adducts

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The measurement of biomarkers is one approach to monitoring presence and effects of toxic chemicals in the environment. DNA adducts, the consequence of covalent damage to DNA, are an important biomarker. However, the amount of DNA in biological samples is small, and the DNA adducts commonly are a trace component of the DNA.

Gas chromatography with electron capture negative ion mass spectrometry (GC-ECNI-MS) is a sensitive form of MS but its scope is limited to volatile, thermally stable compounds. It is most sensitive when a specific ion is formed in high yield. Some DNA adducts arising from simple alkylation damage to DNA can be determined by GC-ECNI-MS after isolation as modified nucleobases and then electrophoric derivatization (1). This approach fails for most DNA adducts because of the structural variety and lability of their functional groups.

In order to broaden the scope of GC-ECNI-MS for sensitive DNA adduct detection, we are applying or developing chemical transformation techniques which convert the DNA adduct into a product which can then be electrophore derivatized and detected with high sensitivity by GC-ECNI-MS. Our first example was to convert N7-(2-hydroxyethyl)guanine, an ethylene oxide DNA adduct, into a corresponding xanthine with nitrous acid. The xanthine form of the adduct was then easier to electrophore derivatize than the parent compound (2). As a second example, we used high temperature (160°C) hydrazinolysis to release 2-aminofluorene from the DNA adduct, N-(deoxyguanosin-8-yl)-2-aminofluorene (3). The 2-aminofluorene was then converted to N-pentafluorobenzylidenyl-2-aminofluorene, a sensitive analyte for GC-ECNI-MS.

Here we introduce potassium superoxide to achieve a third kind of chemical transformation reaction for DNA adducts. The proposed overall method is shown in Fig. 1. Benzo[a]pyrene diol-epoxide is known to react with DNA onto the N2 position of guanine and the subsequent adduct, shown as the starting material in this figure, can be released from the DNA by mild acid hydrolysis (4). Our analytical strategy is to conduct this known step, followed by oxidation of the released benzo[a]pyrene tetrahydrodiol with potassium superoxide, yielding 2,3-pyrenedicarboxylic acid. This diacid is then converted by pentafluorobenzyl bromide into a corresponding diester for detection by GC-ECNI-MS. The detection of 9.2 fg (14 amol) of a standard of this compound by this technique is shown in Fig. 2. The high sensitivity reflects the known tendency of pentafluorobenzyl esters to undergo dissociative electron capture yielding a carboxylate ion in high yield.

Chemical transformation in chemical analysis tends to give up some structural information about the analyte, and thereby some specificity in the analysis. A compensating advantage is that a given method may be applicable to an entire class of analytes. For example, we anticipate that the KO2 procedure will be a general method for diol-epoxide polyaromatic DNA adducts. We have demonstrated previously that a diversity of hydroxy- and keto-polycyclic hydrocarbons can be converted by KO2 into corresponding polyaromatic carboxylic acids (5).

Acknowledgements

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References


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Figure 1. Chemical transformation/electrophoresis derivatization of a benzo[a]pyrene diolepoxide DNA adduct, giving 2,3-bis(pentafluorobenzyl)pyrenedicarboxylate.

Figure 2. Detection of 9.2 fg (14 amol) of a standard of 2,3-bis(pentafluorobenzyl)pyrenedicarboxylate by GC-ECNI-MS (Hewlett Packard 5890 GC, 5988A MS).
APPLICATION OF GC RETENTION INDICES FOR ISOMER SPECIFICITY IN GC/MS MONITORING OF HALOGENATED DIOXINS

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Brominated dioxins (PBDD) have been found in flame-retarded thermoplastic resin pyrolysates, brominated phenols, brominated diphenylether pyrolysates, and vehicular exhaust. Bromochlorinated dioxins (PBCDD) have been detected in municipal and hazardous waste incinerator fly ash. Toxicological data suggest that the toxicities of these brominated and bromochlorinated dioxins (75 PBDD, 1550 PBCDD) are similar to the levels found for the 75 PCDD analogs. Because of toxicity concerns and the scarcity of analytical standards, environmental monitoring for halogenated dioxins (HDD) is usually performed with only a few standards. Because toxicity is dependent upon substitution pattern, isomer-specific analytical methodology would be desirable for HDD, based upon the traditional combination of GC retention time or RI and mass spectrometric data. For quality assurance, it is important that the retention time ranges be known accurately for each congener group, so that GC/MS selected ion monitoring (SIM) descriptors will be assigned the proper retention time ranges. It is also important that the GC column resolve 2,3,7,8-substituted isomers from the close-eluting isomers, and that those RI be well-defined, so that quantifications of the 2,3,7,8-substituted isomers will be accurate.

For this study, commercially-available standards were augmented by the synthesis of over 100 HDD. Retention indices (RI) for the HDD were determined against the normal hydrocarbons, using temperature programmed capillary column gas chromatography. Retention indices for the HDD were expressed as the sum of five contributors: (1) the index of single-ring A; (2) the index of single-ring B; (3) the index of a "ring interaction" effect, or "1,9-effect" which was observed in cases where both the 1 and 9 positions were halogenated. In the event the 1,4,6, and 9 positions all were halogenated, the total effect was experimentally observed to be approximately 1.5 times the magnitude of a single 1,9 effect; (4) the index increment from "buttressing" the 1,9-effect with additional substitution at the 2 and 8 positions; and (5) the index of a "phase interaction" effect. The single-ring RI values are listed in Table 1. In general, each additional bromine substitution incremented the molecular RI about 1.5 times as much as a chlorine substitution did. The 1,9-chloro ring interaction was found to average 12 RI units; when two 1,9-effects were present (i.e. all four 1,4,6,9 positions were occupied by chlorine atoms), the total ring-ring effect was approximately 18 units.

The 1,9-dibromo ring-ring interaction was also found to increase the RI by approximately 12 RI units. Buttressing at the 2,8-positions with chlorines increased the magnitude of the effect to 18 RI units. "Buttressing" the 1,9 positions with 2,3,7,8-tetrabromo substitution increased the 1,9- ring-ring interaction effect to 34 RI units; 2,8-dibromo-3,7-dichloro buttressing gave the ring-ring interaction effect a magnitude of 22 RI units.

In those molecules containing two or more bromines on one ring, it appeared that the ability of the second ring, if unsubstituted, chlorinated only, or monobromo-monochlorinated, to interact with the column phase was diminished, resulting in a reduction of RI. It is suggested that the reduction in RI was related to the molecule assuming a distance from the phase greater than the optimum distance for interaction with the phase by the second ring. The experimental magnitude of the RI decrease, suggested to be caused by a "phase interaction" (PI) effect, was found to be dependent upon the number of bromines and upon whether the second was unsubstituted, chlorinated, or monobromo-monochlorinated. These findings have been summarized in Table 2.

In this study, 69% of the 71 HDD used to test the model had experimental RI values which agreed with the predicted RI values within 3 RI units, over a RI range of ca. 2500-4300 units; 89% were within 5 RI units, and no predictions varied from the experimental values by more than 7 RI units.

NOTICE
Although the research described in this article has been funded wholly or in part by the Environmental Protection Agency contract 68-02-0048 to Lockheed Engineering & Sciences Company, Inc., it has not been subject to the Agency's review and therefore does not necessarily reflect the view of the Agency, and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute an endorsement for use.
Table 1. Single Ring Retention Indices for HDD.

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Table 2. Phase Interaction Effect Values in RI Units

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The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Results of a Field Evaluation of the Bruker Mobile Mass Spectrometer Under the SITE Demonstration Program

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The Superfund Program was established by Congress in 1980 to identify, prioritize, and remediate the nation's uncontrolled hazardous waste sites. Because the scope of the problem has proven to be even more complex and widespread than anticipated, Congress enacted the Superfund Amendments and Reauthorization Act of 1986 (SARA). Under SARA, the U.S. Environmental Protection Agency (EPA) was charged with effecting more timely and cost-effective solutions for Superfund site remediations. As part of this legislation, the Superfund Innovative Technology Evaluation (SITE) Program was established to provide EPA with a program designed to accelerate the development, demonstration, acceptance, and use of promising alternative or innovative technologies targeted to meet the objectives of the overall Superfund Program.

One element of this program provides developers with the means to demonstrate innovative technologies that could be used as an alternative to the current systems for detecting and assessing the extent of pollution at hazardous waste sites. The purpose of these demonstrations is to evaluate fully developed technologies, thereby making information on the performance and cost effectiveness of such technologies available to interested parties. Superfund decision makers will thus have the information necessary to consider whether or not these technologies can or should be used in specific site characterization or remediation projects.

Bruker Instruments, Inc., of Billerica, Massachusetts, was selected to participate in this program, and its Mobile Environmental Monitor (MEM) was identified by EPA as a promising candidate for a field demonstration. The MEM, designed for the on-site analysis of organic contaminants, is a quadrupole mass spectrometer (MS), optionally coupled to a gas chromatograph (GC) or a thermal desorption sampling probe. Currently, full-sized, non-mobile laboratory GC's/MS's have been the preferred EPA approach to identifying and quantifying organic contaminants at Superfund sites. A GC/MS instrument provides information regarding the molecular weight, retention time, and characteristic fragmentation pattern of organic compounds. The primary disadvantages of conventional GC/MS systems for field use are instrument size, power demand, and sensitivity to external factors (e.g., temperature, humidity, and vibration). The development of the MEM, which is rugged enough to withstand a variety of field conditions, is of considerable interest to parties responsible for contaminant monitoring at Superfund sites. Newly developed mobile systems, such as the Bruker MEM, appear to have attained satisfactory levels of stability, power usage, and compactness for field applications and are appropriate for testing under the SITE Program.

The purpose of the demonstration was to evaluate the ability of the MEM to analyze polychlorinated biphenyls (PCB's) and polynuclear aromatic hydrocarbons (PAH's) in soils and to analyze volatile organic compounds (VOC's) in water under field conditions at Superfund sites. The demonstration focused on the capability of the instrument to generate rapid, cost-effective, and reliable PCB, PAH, and VOC data from real-world samples. The experimental design used in this study was to compare MEM performance to analytical method performance results as would be received under the EPA Contract Laboratory Program (CLP). Detailed project and quality assurance (QA) plans were prepared which defined the sampling and analysis protocols, the experimental design, the QA and quality control (QC) requirements, the data base management system, the health and safety considerations, and the proposed methods for data analysis.
For this demonstration, samples were collected from two sites in Massachusetts (EPA Region 1) on the National Priorities List (NPL). These sites were selected on the basis of documented (Record of Decision) presence of analytes of interest: i.e., PCB’s in soil and VOC’s in ground water at one site and PAH’s in soil at the other. A screening analysis by the MEM identified the collection points (i.e., low, medium, and high concentration levels) for five samples in each compound class (PCB, PAH, VOC). Bulk samples were collected, homogenized, and split into replicates. For each compound class, each of the distinct samples was split into seven replicates for analysis on site (or near site) by the MEM, and off site by standard EPA methods. In addition, standard reference materials and blank samples were sent to all analysis locations as a measure of variability, detection limits, and other data quality assessment criteria. This process worked well for the PCB and PAH soil samples; however, remediation activities at the chosen site precluded the collection of VOC-contaminated ground water samples. Instead, a surface water sample was collected from one site and spiked at different concentrations of VOC’s. These samples were then analyzed under field and laboratory conditions as originally planned.

The data from all analysis sites were compiled into one fully documented data base. Data were then subjected to a detailed verification process. Following verification, a variety of data analyses were performed, including intermethod comparisons (between the MEM and the off-site laboratory results), reproducibility was estimated (from replicate analyses on the same instrument), and an evaluation of various data quality indicators was made. Direct comparison plots and a variety of statistical routines were used to interpret the data.

The primary advantages of the MEM are its portability and ruggedness. Rechargeable batteries supply all power required, and the logistical requirements are minimal and easily fulfilled. The use of purified ambient air as the carrier gas eliminates the need to transport compressed gas cylinders. The operations plan called for the analysis of 13 samples per day, which is within normal performance expectations; however, the analysis team had difficulty meeting this sample throughput requirement. Although the MEM is easy to operate under normal conditions, a skilled operator is required to correctly diagnose and repair malfunctions.

On the basis of data collected and observations made during this demonstration, several issues have been identified that must be addressed before the MEM or other GC/MS instruments can be recommended for Superfund site monitoring, characterization, and remediation activities. These issues include: (1) method enhancement and procedural requirements, (2) development of standardized QA/QC requirements, (3) development of data reporting standards for field analytical measurements, and (4) development of detailed troubleshooting guides and training programs. These issues do not necessarily represent problems with the technology itself; several are external factors or policy issues that require attention before the EPA can use field mobile GC/MS instruments as reliable field analytical devices.

EPA is conducting additional mobile mass spectrometer research at the EMSL-LV under the SITE Program. Future laboratory evaluations will concentrate on (1) separating variability associated with the instrument from that associated with the methods, (2) formalizing QA/QC procedures, and (3) establishing consistent data reporting procedures for field applications. Additional field demonstrations and evaluations will follow.

NOTICE

Although the research described herein has been funded wholly or in part by the U.S. Environmental Protection Agency under Contract Nos. 68-03-3249 and 68-CO-0049 to Lockheed Engineering & Sciences Company, it has not been subject to Agency review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.
THE ULTRA-TRACE DETERMINATION OF LSD BY LC/MS/MS WITH ION FORMATION AT ATMOSPHERIC PRESSURE.

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Social and analytical problems still remain with respect to LSD. Its identification in forensic samples continues to be an analytical challenge utilizing modern GC/MS techniques. Recent experience regarding confirmation of urine RIA positives collected within the military suggests that only about 10-30% of these samples can be confirmed by GC/MS techniques. This problem stems at least in part from sensitivity differences between RIA screening and GC/MS confirmation techniques. Confirmation of relatively low levels (sub-ppb) that LSD exists in these urine samples demands sensitivity requirements that challenges current GC/MS standards. A factor that contributes to the GC/MS confirmation problem includes the high polarity and thermal instability exhibited by LSD. An ultra-trace derivatization procedure prior to analysis of the urine extract is thus required. The chemical complexity of urine extracts adds to the analytical problems and hinders unequivocal identification of this important drug and its metabolites.

We have explored the use of both the ion spray (pneumatically-assisted electrospray) and the heated pneumatic nebulizer (APCI) LC/MS interfaces for the ultra-trace determination of underivatized LSD. A comparison of LC/MS results on the commercial SCIEX API III and a modified Hewlett-Packard 5970 MSD system using both 2.1-mm- and 1-mm i.d. packed-HPLC columns have demonstrated sub-nanogram LC/MS detection limits. This report summarizes our results with an emphasis on the commercial Sciex system optimized for high sensitivity using the ion spray LC/MS interface.

EXPERIMENTAL

A Sciex TAGA 6000E triple quadrupole mass spectrometer upgraded to an API III was used in this work. The system was operated in the selected reaction monitoring (SRM) mode using argon as the collision gas with a collision energy of 20 eV (laboratory frame) with positive ion detection mode. The HPLC system used included a 600 MS pump (Waters Chromatography Division, Milford, MA) operated in the isocratic mode at 0.1 mL/min with 35:65 acetonitrile:water with 5 mM ammonium formate as the sluent. The column was a 2.1 mm x 50 mm LC8-DB (Supelco, Bellefonte, PA) equipped with a Model 7125 injector (Rheodyne, Cotati, CA). Human urine samples (5 mL, pH-adjusted to 10) were treated using a simple n-butyl chloride liquid-liquid extraction procedure, and the extract analyzed without derivatization. Injection volumes of 10-20 μL were analyzed by the LC/MS/MS system. The internal standard was da-LSD spiked into the urine at the 500 pg/mL level. Spiked urines containing 500 pg/mL da-LSD were analyzed containing the following levels of LSD: 0, 20, 50, 100, 200, 500, and 1000 pg/mL.

The corresponding LC/MS/MS mass spectra for LSD, iso-LSD, and da_LSD are shown in Figure 1. The analysis of urine extracts was carried out under selected reaction monitoring (SRM) conditions that included three Ions for LSD and two ions for the internal standard. The conditions described were chosen to represent a combination of high sensitivity and practical utility.

RESULTS AND DISCUSSION

The results of this work demonstrate that LC/MS/MS analyses of human urine extracts may be be accomplished with short sample analysis times and LSD levels at least as low as 50 ppt. For example, the extract from 5 mL urine may be concentrated to 50 μL while 20 μL of this were injected onto the 2.1 mm i.d. column. Isocratic HPLC conditions allow elution of underivatized LSD and its metabolites within 6 min. Structurally characteristic product ions plus the precursor ion and the corresponding HPLC retention time allows unique specificity for the detection and, in principle, quantitation, of LSD in human urines.

An incurred urine sample obtained from an unidentified military personnel was
analyzed by this technique. The described LC/MSMS analysis using SRM and a spike of 500 pg/mL \textit{d}_3\text{-LSD} produced the LC/MS/MS ion current profiles shown in Figure 2. The LSD component is observed at a retention time of 4.2 min while a large peak for iso-LSD is observed at a retention time of 5.8 min. It is worthy of note that these two components are readily detected without derivatization in this experiment. Although it is possible to chromatographically distinguish LSD from iso-LSD by GC/MS following trimethylsilylation derivatization, the described LC/MSMS technique demonstrates good separation and easy differentiation of these compounds within 6 min. Prior analysis by GC/MS/MS following derivatization had determined a LSD level of 370 pg/mL and an iso-LSD level of 1000 pg/mL in this urine. Comparison of relative peak areas from these LC/MS/MS results revealed a quantitative determination of 389 pg/mL for LSD and 940 pg/mL iso-LSD, in good agreement with the GC/MS/MS results.

**CONCLUSIONS**

At the present time the 50 pg/mL level appears to be the practical limit of detection using this technique, but it appears this can be improved to at least the 20 pg/mL level. If a cut-off level of 100 pg/mL LSD is established for regulatory matters the present 50 pg/mL should be sufficient. However, an alternative deuterated internal standard is needed that has abundant product ions differing from LSD.

![Figure 1](image1)

![Figure 2](image2)
CONFIRMATION OF AN INCIDENT OF DIARRHETIC SHELLFISH POISONING IN NORTH AMERICA BY COMBINED LIQUID CHROMATOGRAPHY IONSPRAY MASS SPECTROMETRY

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Diarrhetic shellfish poisoning (DSP) is a severe gastrointestinal illness caused by the consumption of shellfish contaminated as a result of feeding on toxic dinoflagellates [1]. While bivalve contamination by DSP toxins is particularly acute in Japan and northern Europe, there have been no confirmed reports of DSP incidents in North America. The toxins associated with DSP are lipid-soluble, polyether compounds which are difficult to analyze by conventional methods. Recently we have developed a combined liquid chromatography atmospheric pressure ionization mass spectrometry (LC-MS) method using an IonSpray (ISP) interface for the analysis of DSP toxins and reported the presence of okadaic acid (OA), the principal toxin associated with DSP, in natural populations of Dinophysis spp. from eastern Canadian waters [2].

In early August 1990, several people developed symptoms of nausea, vomiting and diarrhea after eating cultured mussels from the Mahone Bay area in Nova Scotia. DSP was suspected and extracts from samples of raw mussels from a restaurant and cooked mussels from a domestic residence were found to be toxic to mice (IP injection). Analysis by an improved gradient elution LC-API-MS method using selected ion monitoring quickly established the presence of a high level of the DSP toxin, dinophysistoxin-1 (DTX-1, a methylated homologue of OA), in the suspect mussels and not in control mussels (Figure 1). Interestingly, OA was not detected. The formation of chemical derivatives which gave the correct shifts in both retention time and mass by LC-API-MS provided additional supporting evidence. The toxin was subsequently isolated, purified and its structure confirmed by a variety of analytical techniques including full-scan ISP-MS, derivatization and HPLC with fluorescence detection, high resolution FAB-MS and proton NMR.

Extraction and handling procedures were improved during the investigation, allowing the quantitative analysis by LC-API-MS of survey samples from the affected area. It was found that the incident was highly localized to one mussel growing lease and that by the end of August, the mussels had depurated all of their toxin load. The most toxic mussels, containing up to 1000 ng/g whole tissue (the legal level in Europe is 200 ng/g), appear to have been harvested on August 3. This suggests that a bloom of toxic plankton may have occurred near the end of July and led to contamination of the mussels.

Microscopic examination of toxic mussel digestive gland contents revealed remnants of Dinophysis spp. (probably norvegica), an organism known to produce both OA and DTX-1 (although the ratio is known to vary, with documented instances in Norway where only DTX-1 is present [3]). Control, non-toxic mussels showed no recognizable remnants of Dinophysis spp. Plankton tows from the water adjacent to the affected area in mid-August did show several Dinophysis spp. (including norvegica as the dominant species), but at low levels in the water column. LC-MS analysis of these plankton samples provided only a weak, unreliable signal for DTX-1, and it is presumed that the plankton bloom had dissipated by the time of sampling.

To our knowledge this is the first proven case of DSP in North America.

\[
\text{Dinophysistoxin - 1 (DTX-1)}
\]

\[
\begin{align*}
\text{a) Standards} \\
\text{m/z 805 + 819}
\end{align*}
\]

\[
\begin{align*}
\text{b) Suspect mussel extract} \\
\text{DTX-1}
\end{align*}
\]

\[
\begin{align*}
\text{c) Control mussel extract} \\
\text{DTX-1}
\end{align*}
\]
Particle beam LC-MS Using The Jeol SX-102 Mass Spectrometer. Woodfin V. Ligon, Jr. and Steven B. Dorn, General Electric Co. Corporate Research and Development, Schenectady, N.Y. 12301

We have described the design and construction of a particle beam LC-MS interface specifically designed for use with magnetic mass spectrometers. (Ligon and Dorn, Anal. Chem., 62, 2573, (1990)). This early work utilized a Varian-MAT 731 which was relatively insensitive, slow scanning, and allowed only EI analysis. We have now successfully transitioned this interface to a JEOL SX-102 mass spectrometer. Using this instrument we have demonstrated sub-nanogram sensitivity using one second mass scans (40-800 amu) in EI mode. In addition, we have developed methods which allow acquisition of CI data at full accelerating voltage (10 keV) with excellent sensitivity using a wide range of reagent gases. When operating with an LC flow of 0.5 ml/min acetonitrile and isobutane reagent gas, the base pressure in the ion source region is about 2 x 10^{-6} torr. Because of the very low pressures obtained with our interface, the operation of the mass spectrometer is not compromised in any way. We have demonstrated detection of masses above 2000, high resolution scanning and high resolution SIM.

Figure 1. Selected ion monitoring of cholesterol at 10,000 resolution

<table>
<thead>
<tr>
<th>SUB</th>
<th>THEORETICAL</th>
<th>EXPERIMENTAL</th>
<th>ERROR (ppm)</th>
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<tr>
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<td>0.4</td>
</tr>
<tr>
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<td>386.3840</td>
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<tr>
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<td>386.3849</td>
<td>386.3871</td>
<td>2.2</td>
</tr>
<tr>
<td>C_{27} H_{46} O</td>
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<td>2.0</td>
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<tr>
<td>C_{27} H_{44}</td>
<td>386.3842</td>
<td>386.3840</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Figure 2. Mass measurement accuracy for cholesterol using 1.4 second scans at 10,000 resolution.
Figure 3. Total ion current and selected ion chromatograms for the GPC analysis of a methyl silicone fluid having an average molecular weight of 2000. Ionization was by ammonia chemical ionization.

Figure 4. Averaged ammonia CI mass spectrum obtained from the experiment in Figure 3. The average was taken over the entire elution of the silicone fluid.

\[(\text{CH}_3)_3\text{SiO-}[\text{Si(\text{CH}_3)_2\text{O}]_n\text{Si(\text{CH}_3)_3}\]
The Determination of Hydroperoxides, Peroxides and Other Labile Compounds by Particle Beam EI-MS/MS. Richard Hiserodt, Ming-Hua Yang, Thomas G. Hartman, Joseph Lech, Tarik Roshdy, Karen Schaich and Robert T. Rosen*. Center For Advanced Food Technology and Food Science Department, Cook College, Rutgers University, New Brunswick, NJ 08903

The inertness of the particle beam technique was highlighted in this presentation by showing EI spectra of the most sensitive and labile organics, hydroperoxides and peroxides. The compounds decompose with heat and by metal catalysis. Use of PEEK columns and tubing with HPLC, and the particle beam interface with EI-MS overcomes this disadvantage and spectra were presented of compounds which cannot be analyzed by any other MS technique. Lipid peroxidation products such as linolenyl hydroperoxide have been postulated as intermediates for years, and their decomposition pathways lead to flavors as well as toxic polymers. EPR has been the only standard of analysis for years. Now methodology is possible by both HPLC and HPLC-MS.

The EI-mass spectrum of a mono hydroperoxide of methyl linoleate was shown. Characteristics ions in this mass spectrum are the odd electron ions at $m/z=292$ and $m/z=308$. These result from loss of $H_2O$ and $H_2O_2$ from the molecular ion. The low mass ions for this compound consist of a series of ions separated by methylene units and are characteristic of linolenic acid, the analog with three double bonds. Also shown was the EI-spectrum of a dihydroperoxide of methyl linoleate. The primary fragments at high mass which characterized this spectrum include the ion at $m/z=325$ resulting from loss of $OOH$ from the molecular ion. The molecular ion can also lose a molecule of $H_2O$ to form the ion at $m/z=324$ which immediately loses $O$H and $OOH$ to form the ions at $m/z=307$ and $m/z=291$. Loss of a molecule of oxygen produces the ion at $m/z=316$ which can lose $O$H or $OOH$ to form the ions at $m/z=309$ and $m/z=293$ respectively.

Work was also done using this technique to determine the applicability to other peroxides. The EI-spectrum of 1-(hydroperoxycyclohexyl)dioxydicyclohexanol did not show a molecular ion nor did we detect ions for loss of $H_2O$, $H_2O_2$, $OH$, or $OOH$. The even electron at $m/z=115$ is formed by inductive cleavage at the peroxide linkage. Loss of $H_2O$ from this ion yields the even electron ion at $m/z=97$ and loss of ethylene from this ion yields the even electron ion at $m/z=69$. Triphenylmethylhydroperoxide did not show a molecular ion nor did we detect ions for loss of $H_2O$ or $H_2O_2$ but we did detect a ion at $m/z=259$ due to loss of $OH$ from the molecular ion. We detected the trityl carbocation at $m/z=244$. Triphenylmethylhydroperoxide did not show a molecular ion nor did we detect ions for loss of $H_2O$ or $H_2O_2$ but we did detect a ion at $m/z=244$ an odd electron ion and may result from an McLafferty rearrangement to the ionized ring with subsequent loss of a molecule of oxygen by alpha cleavage. Inductive cleavage of the ionized oxygen closest to the ring in ((2,3,5,6-tetramethyl-1,4-phenylene) dimethylenebis(t-butyl peroxide) yields the ions at $m/z=249$ and $m/z=73$. Loss of the second t-butyl group yields the ion at $m/z=176$, or $m/z=175$ if it is lost as t-butyl alcohol.

Solutions of these hydroperoxides and peroxides were prepared in acetonitrile and chromatographed on a Syncropack B10-C RP-P (C-18) HPLC column. The mobile phase consisted of water with varying amounts of acetonitrile. The samples were also chromatographed using normal phase conditions on a silica column using a mobile phase consisting of hexane / ethyl chloride / ethan. The hydrophobic nature of the hydroperoxides and their precursors indicate normal phase conditions to be preferable.

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PARTICLE BEAM LC-MS SPECTRA OF QUATERNARY AMMONIUM SURFACTANTS

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The ability of particle beam (PB) LC-MS to provide useful analytical information for nonvolatile cationic surfactants is demonstrated. These compounds, usually in the form of quaternary ammonium salts, are often the primary active ingredient in hair conditioning products. In this application they are usually of the types

\[ R_1 N(CH_3)_3^+ X^- \] and \[ R_1 R_2 N(CH_3)_2^+ X^- \]

where the alkyl chain, \( R \), varies from 10 to 22 carbons. Analytical requirements for these materials usually include determination of the number and lengths of alkyl chains, and the chain length distribution.

We find that PB vaporization processes of these salts preserve the structural integrity of the analytes and that EI and CI techniques meet most analytical requirements. Use of PB-MS for this type of work has the advantage of allowing easy changeover to non-surfactant or even non-LC applications.

Figure 1 illustrates a typical EI spectrum for a monoalkyl quat. The ion at m/z 368 corresponds to flash desorption of the intact quaternary ion. The m/z 353 ion is the molecular ion of the tertiary amine formed by pyrolytic demethylation. Its intensity relative to the m/z 58 immonium ion is consistent with the normal EI spectra of long chain alkyl dimethyl amines. The methane chemical ionization spectra (not shown) are dominated by \( MH^+ \) and \( M-H^+ \) ions of this tertiary amine with much smaller amounts of the intact quat. A third feature of the EI spectra of these types of compounds is a series of fragment ions that correspond to alkane losses from the intact quat ion (m/z 352, 338, 324, 310, 296, ...). These appear to be due to charge-remote fragmentations occurring in the ion source. This mechanism has previously been seen in CID MS/MS spectra of these compounds. These features persist in the homologous series of C12 to C22 monoalkyl quats and chain length distributions can be determined from the molecular ion intensities.

Figure 2 illustrates typical EI and CI spectra obtained for dialkyl quats. Negligible amounts of intact quats are seen but dialkyls are easily distinguished from monoalkyls because \( \alpha \)-fission from the dealkylation amine product now produces a stable medium mass immonium ion with retention of the second alkyl chain. In addition the CI spectra exhibit...
intense hydride abstraction ions from the dialkylmethyl tertiary amine. Chain length and distribution are determined from either the medium mass fragment (EI) or the molecular ions (CI).

The competition between desorption and dealkylation seen in monoalkyl guats provides an opportunity to test the effect of ion source temperature on these volatilization mechanisms. A plot of the ratio of relative intensities (% TIC) of intact guat ions to tertiary amine molecular ions (masses 284 and 269 for hexadecyltrimethylammonium chloride) (Fig. 3) shows a threshold behavior with subsequent increase in intact guat desorption up to the 300 °C limit of our ion source.

These results indicate the PB volatilization processes seen here generate species which are directly related to the structure of the original nonvolatile salt and that increasing source temperature improves the likelihood of observing intact quaternary ions. This work was done on an Extrel ELQ 400-1 MS with the Thermabeam PB interface. The LC flow was usually about 0.6 ml/min of 50/50 water/methanol.
Sensitivity and ionization softness are used to gauge the settings for a particle beam LC/MS interface. The sensitivity is a measure of the relative efficiency with which the particle beam delivers the solute into the ionization chamber and the ionization softness a measure of the relative ease of delivery and subsequent ionization. Some of the variables that may influence the solute delivery include the mobile phase composition and flow rate, the choice of nebulizer and its flow pressure, the position of the fused silica tube in the desolvation chamber, the nature of the compounds being analyzed, the addition of volatile salts, the pumping capacity, the size of the skimmer in the momentum separator and the ion source temperature[1]. Normally, the optimization procedure involves adjusting each variable independent until a perceived optimum is reached. As with all optimization schemes this approach is subject to faults optimum. Additional, one may never reach the true optimum using the "one variable at a time" approach if the variables interact. There are several methods for optimizing a process including simplex algorithms and experimental design. A designed experiment is used to understand the process and map the relationships among the variables within an operating space through math models.

In prelude to running the experiments, all of the known variables were considered and evaluated for appropriateness for inclusion in the model. Some of the variables pertaining to the LC analysis method and the interface design characteristics were treated as constants. Under normal circumstances the LC method would be optimized for solute resolution. The organic mobile phase and LC flow rate were considered uncontrolled as far as the mass spectrometer in concern and were held constant during these course of the experiments. Likewise the pumping capacity, the position of the fused silica tube in the desolvation chamber, the skimmer size and the ion source temperature where treated as constants. Consequently, only five variables such as Acetonitrile mobile phase composition, second stage nebulizer flow pressure, nebulizer temperature, desolvation chamber temperature, and the ammonium acetate concentration in the mobile phase were evaluated for their effects on Extrel's ELQ-400-3 QQQ LC/MS system. P-methoxy-1-phenyl acetic acid (M.W. 166) was used as a model compound for this study. Ion source temperature and the LC flow rate were set at 280°C and 0.4 mL/min respectively throughout this work. The first series of experiments were based on a D-optimal design with inclusions to allow the examination of the main effects, interactions, and some of the quadratic terms. There were 74 points included in the model with each data point being an average of 3-5 replicates. The data were analyzed using the computer program RS/Discover running on a VAX 6410.

The results fit the quadratic model better than a simple interaction model. Figure 1 shows a normal probability plot of the residuals when a quadratic model is used. Note that some data points still deviate from the line, suggesting that the relationship between the response sensitivity and all five variables is not purely quadratic. The main effects found are nebulizer flow, nebulizer and desolvation chamber temperatures as shown in Figure 2, where the effects with 95% confidence level are plotted. The effect of salt on the response does not appear to be significant in this model. However, the effect of salt is coupled with desolvation chamber temperature, especially when the temperature is high (Figure 3). Other two-term couplings are found between the mobile phase composition and nebulizer flow, and between nebulizer flow and its temperature (see the low number on the "signif" column in Table 1). The relationship between the ionisation softness, measured by the area ratio of m/z 166 to m/z 121, and these five variables has not been analyzed by an appropriate model yet. The raw data suggests that the softness increase with the response sensitivity (Figure 4).
Supercritical fluid chromatography fills an important role in many industrial laboratories in the characterization of less-volatile, nonpolar mixtures. One goal of our research has been to provide on-line SFC-MS support for other SFC users, most of whom use capillary SFC, unmodified CO$_2$ as the mobile phase, and flame ionization (FID) or ultraviolet detection. We most generally use the direct-fluid-introduction interface in our SFC-MS work. In this interface, the entire capillary-column effluent is introduced directly into the ion-source ionization region. Unfortunately, we, as well as other researchers in the field (1), observe a substantial drop in SFC-MS signal-to-noise ratio (S/N) and an increase in fragmentation above SFC pressures of 300 to 350 atm, at typical capillary-column flow rates. This became a real limitation after the upper pressure limit of many of our SFC systems was increased to 560 atm (2).

We've used simple, liquid-nitrogen-cooled cryopumps to increase the pumping capacity of our triple-quadrupole mass spectrometer. This has made a dramatic improvement in the SFC-MS S/N and the spectral quality at higher SFC pressures. Previously, we presented our preliminary results (3). Since that time, we've continued our investigations of cryopumping and have made significant advances. The optimized cryopump arrangement consists of one cryopump in the ion-source-manifold region and two cryopumps in the analyzer-manifold region. Of the latter pair, one lies over the first quadrupole mass analyzer (Q1), while the second lies over Q3.

Figure 1 shows a plot of analyzer pressure vs. SFC pressure using the optimized cryopump arrangement. At an SFC pressure of 550 atm in the CI mode, the analyzer pressure is approximately 8 x 10^-6 torr with cryopumping (2 x 10^-3 torr before our most recent modifications), while it reaches nearly 7 x 10^-5 torr without cryopumping.

We've used a variety of samples as probes during our investigation of cryopumping. For example, we observed a 10-fold improvement in the abundance of the ammonium adduct ion of the oligomer of poly(ethylene) glycol with an ethoxylate value of 35 over our previous best results.

It has become increasingly clear that cryopumping consistently improves SFC-MS performance above 300 to 350 atm. Cryopumping has enabled us to routinely provide both electron and chemical ionization spectra through the full SFC pressure range. In fact, since we began using cryopumping we have not yet encountered an analyte which can be eluted and detected by capillary SFC-FID which we cannot likewise elute and detect by SFC-MS. The cryopumps are simple to use. They are filled with liquid nitrogen before a series of runs and "topped off" 2 to 3 times during the course of a day. The cryopumps warm during the night and the condensed CO$_2$ is pumped from the cryocooled surfaces. The use of the cryopumps is so straightforward and their benefits so clear that we now use cryopumping on a routine basis for all our SFC-MS work.

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Figure 2 illustrates our ability to now obtain a peak-to-peak match between the SFC-FID chromatogram and the SFC-MS reconstructed-total-ion-current chromatogram. Figure 2 compares the chromatograms obtained for an
alcohol reaction mixture residue. The first peak is residual octadecanol. The second multiplet consists of fatty-fatty esters and high-molecular-weight alcohols produced by condensation of fatty alcohols. The last multiplet of 6 peaks was not observed during an earlier GC-MS analysis. The methane-CI SFC-MS data revealed these peaks to be esters of the high-molecular-weight condensation alcohols with fatty acids. (Fatty acids were present at low levels in the reaction mixture.) Figure 3 shows the SFC-MS CH₄ CI spectrum of the last peak in the multiplet, corresponding to a 54-carbon ester. The spectrum appears to be a "normal" CH₄ CI spectrum of a fatty-fatty ester, despite the high molecular weight of this species and the presence of a significant quantity of CO₂ in the ion source during ionization. As expected, ions corresponding in mass to the loss of the acid moiety are present.

Unexpected results were obtained during the NH₃ CI SFC-MS run of a series of trimethylsilyl (TMS)-derivatized secondary amines. The apparent masses were 44 Da higher than expected. This suggested that the TMS-derivatized amines were reacting with the SFC mobile phase, CO₂. Such reactions have been observed with primary amines, but we have not observed the reaction of secondary amines with CO₂. We confirmed that the TMS-derivatized secondary amines were reacting with CO₂ by using N₂O as the SFC mobile phase.

References

The potential of pseudo-electrochromatography in LC-MS


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Introduction

Combined liquid chromatography mass spectrometry (LC-MS) is now a well established technique for the detection, quantitation and identification of polar organic compounds. A variety of LC-MS interfaces have been developed, which can be applied in various ranges of analyte polarity. However, due to compatibility problems with respect to the mobile phase composition only reversed-phase LC is coupled to mass spectrometry. As a result of the expanding applicability range of MS, which is due to the introduction of powerful liquid-based soft ionization techniques, i.e., FAB, ESP and TSP, the analysis of ionic compounds by LC-MS methods now becomes feasible. However, the LC separation of ionic compounds is performed using ion-exchange, ion-pair or ion chromatography, where mobile phase are used that are generally not compatible to routine LC-MS operation.

In the past years we have been developing techniques, that can overcome the mobile phase compatibility problems. For target compound analysis, the phase-system switching approach was introduced. However, when the LC-MS analysis of mixtures is necessary, a more versatile approach is required. Pseudo-electrochromatography (\(\psi\)EC) has been developed and investigated for its potential in the LC-MS analysis of ionic compounds. Some important features of \(\psi\)EC in comparison with other separation techniques are given in the Table.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Driving force</th>
<th>Migration</th>
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<tbody>
<tr>
<td>Liquid chromatography</td>
<td>pressure</td>
<td>capacity factor</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>electro-osmosis</td>
<td>electrophoretic mobility</td>
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<tr>
<td>Electrochromatography</td>
<td>electro-osmosis</td>
<td>capacity factor + electroph. mobil.</td>
</tr>
<tr>
<td>Pseudo-electrochromatography</td>
<td>pressure + electro-osmosis</td>
<td>capacity factor + electroph. mobil.</td>
</tr>
</tbody>
</table>

Experimental

\(\psi\)EC is performed using packed microcapillary columns (220 \(\mu\)m ID). A schematic diagram of the on-line combination of \(\psi\)EC and CF-FAB MS is given in Figure 1. A conventional reciprocating LC pump in combination with a pre-injector split is used for solvent delivery. The FAB matrix solution is added in the post-column mode by using a liquid-junction coupling. CF-FAB MS is performed on a Finnigan MAT 90 double focussing mass spectrometer.
Results and discussion

The vEC technique has been used in the analysis of nucleotides using CF-FAB MS. A typical example of the effect of applying a potential over the chromatographic column next to the pressure driven flow is given for the separation of AMP, ADP and ATP in Figure 2.

Conclusions

vEC is readily applicable for the analysis of ionic compounds. It avoids the use of non-volatile mobile phase additives, that are normally required in the separation of ionic compounds. Furthermore, it gives the possibility to further tune both the selectivity in chromatography and the chromatographic performance in terms of efficiency. Presently, 10-50 pmol full-scan detection for nucleotides in CF-FAB have been obtained. vEC shows great promise for future application in the LC-MS analysis of ionic compounds.

Future work aims at a more fundamental description of vEC as well as the use of voltage ramping during the chromatographic run. It is expected that continuously changing the potential during the run will give even more powerful ways to control the separation.
A NEW PROBE INSERT FOR A THERMOSPRAY INTERFACE THAT ALLOWS RAPID REPLACEMENT OF CLOGGED PROBES AT NOMINAL COST

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One of the major problems encountered when operating a thermospray interface is the continual clogging of the approximately 100 micron opening in the vaporizer tip. The reason this tip clogs frequently is because of the nature of the thermospray process. The thermospray vaporization process deposits involatile species at the tip which degrade the performance and eventually clog the tip. There are other mechanisms that, also, lead to clogging. For example, if the solvent or analyte contain particles 100 microns or larger, they can lodge in the probe and clog it. To this end .2 micron filters are installed on the inlet to the probe. In the author's lab, compounds have been run which polymerize at about 200C. Analyzing these compounds can result in replacement of the probe 3 or 4 times in one day due to the polymerization reaction occurring near the tip. Once the probe has become clogged, one can try removing the clog by chemical means, but this author has never had much success with those methods. The design adopted by a number of instrument companies involves replaceable probe tips which can be removed and cleaned or discarded. If replacement of the tip does not solve the problem, then one must replace the entire probe. These probes are generally expensive and, if probes have to be replace a few times a day, the cost becomes prohibitive.

There have also been a number of designs in the literature which claim to solve the clogging problem. One uses laser drilled apertures which can be reused after cleaning. The insert closest to the design described in this paper has a very simple and straight forward probe replacement. The replacement requires the spot welding of the TCI and TC2 thermocouples onto a 1/16 inch piece of capillary tubing which is then inserted through a modified Swagelok fitting into the vacuum chamber.

The two probes that the author designed are similar to each other and are shown in Figures 1 and 2 respectively. With these designs no spot welding is needed when replacing the probe inserts, and the capillary tubing is supported into the probe handle to minimize vacuum leaks. In the first design, a 1/16 inch stainless steel tube with .005 inch I.D. (127 micron) is used to vaporize the sample. In the other design, a .21mm polyimide clad quartz capillary is used. The beauty of this technique is that when a probe clogs the old tubing is simply removed and for the price of two ferrules and a short piece stainless steel capillary tube ($10.00) the probe is repaired.

In the case of the stainless steel capillary, one can either heat the tube directly by placing the electrical connections on the 1/16 inch tubing or indirectly by placing the electrical connection on the guide tube. One might expect there to be a difference between heating directly and indirectly, but no difference could be observed. The quartz capillary could only be heated indirectly due to its lack of conduction.
The performance of the two probes was satisfactory, but the quartz capillary tended to be a little less stable than the stainless steel capillary. This instability was probably due to small vibrations near the tip caused by the evaporation of the solvent. The replacement of the quartz tube was also less convenient because of the very narrow diameter and the need for keeping it clear of obstructions. When compared to the commercially available probes from Kratos, the two probes were very similar even though the thermocouples are not directly mounted on the capillary that transfers the fluid and one might expect very sluggish responses to changes in temperature which would lead to less ion signal. In fact, the total ion currents with the new probes are as high if not higher than those obtained with the commercial probes.

4. These probes were built by Scientific Instrument Services, Ringoes N. J.
5. Quartz capillaries are available with ID from 25-100 micron.
6.  
VUV PHOTOIONIZATION MASS SPECTROMETRY: RAPID SCREENING FOR DRUGS OF ABUSE IN URINE

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Introduction: A molecular hydrogen laser coupled to a time-of-flight mass spectrometer has demonstrated the ability to detect drugs of abuse in urine with minimal sample pretreatment. The laser output consists of 7.8 eV photons over a series of lines in the vacuum ultraviolet, which produces only parent molecular ions for many drugs and pharmaceuticals. In addition, due to the selectivity of 7.8 eV photoionization, drugs of abuse can be rapidly screened in urine samples by using a simple solid-phase extraction technique for sample clean-up, followed by direct MS analysis of the resulting extract. Photoionization mass spectra of extracts from pooled urine samples spiked with cocaine, codeine, morphine, phencyclidine, and methadone consist of parent molecular ions for the drugs with a few non-interfering ion signals from the matrix.

Experimental: The molecular hydrogen laser source, described previously (1,2) is a Blumlein discharge apparatus with a narrow discharge channel (330 μm) and a low-inductance spark gap switch purged with nitrogen. Voltage on the bottom Blumlein plate was 20 kV and a hydrogen pressure of 45 torr was maintained in the discharge channel. The laser was operated at a duty cycle of 10 Hz and has an average peak pulse energy of 30 μJ. For mass analysis, a linear time-of-flight mass spectrometer (3) was used, consisting of a 1.5 m drift tube with a Galileo Electro-Optics Z-stack microchannel plate detector (10^8 gain) mounted at the end. A LeCroy 9400A digital oscilloscope was used to store mass spectra averaged over 200 laser pulses and the data was then transferred to an IBM compatible 286 computer for analysis.

Urine samples were pooled from five healthy male graduate student volunteers. 5 ml samples were spiked with μl aliquots of 1 mg/ml drug solutions in methanol. Blank and spiked urine samples were then immediately extracted with Bond Elut Certify (Analytichem International) columns using the manufacturer's suggested procedure (4) for a particular drug class, however, no derivatization steps were applied. Effluent collected from the columns was evaporated to a residue and transferred to sample vials which were then placed in a direct insertion probe for mass analysis.

Results and Discussion: The photoionization mass spectrum of an extract from a urine sample spiked with 20 ppm cocaine is shown in Figure 1. A clear parent molecular ion at mass 303 is observed as well as a fragment ion at mass 182, due to the loss of benzoate. Cocaine was the only abused drug which yielded a fragment ion in the photoionization mass spectrum. For urine samples spiked with cocaine as low as 500 ppb, clear ion signals were recorded above the level of background. Figures 2 and 3 show photoionization mass spectra from extracts of urine samples spiked at 200 ppb with methadone (mass 309) and phencyclidine (mass 243), respectively. In each case, an intense parent molecular ion is observed with some peaks from the matrix which do not interfere with the M^+ signals. Similar results were observed for urine samples spiked at the 200 ppb level with opiates morphine and codeine.

The hydrogen laser/TOF system in its current configuration is capable of drug screening in urine at levels well below cut-off values listed for the technique of enzyme multiplied immunoassay (EMIT). In addition, the technique should be less subject to chemical interferences which produce false test results in EMIT. While background ion signals from the urine matrix are observed in mass spectra for extracts of blank and spiked samples, it is known that electron impact features give rise to background signals in spectra recorded without any sample in the source. These features, such as the low mass signals observed for residual air, are due to electrons which are generated from
interaction of the laser radiation with metal surfaces in the ion source. Currently, the laser radiation is directed into the ion source through a purge tube with two CaF$_2$ windows. Better focusing of the laser radiation into the ion source should significantly reduce the observed background from the urine matrix and improve sensitivity.

References

Reduction of alkyl halides to the trichloromethyl radical by liver microsomal P-450 is the initial event leading to hepatotoxicity. The reductive metabolism of BrCCI₃ by ferrous hemoglobin was used as a model system. This reaction leads to the alteration of the prosthetic heme to form products that can be dissociated from the apoprotein and others that are irreversibly bound to the apoprotein. Proteolysis of the irreversibly bound adduct with elastase overnight at 37°C gave two heme-peptides (HP1 and HP2) that were isolated by reverse phase HPLC, using a C₁₈ Vydac column. These were characterized by Edman sequencing giving the following amino acid residues C-D-K-L-H (residues 93-97 of the hemoglobin β-chain), positive ion plasma desorption mass spectrometry (PDMS) (figure 1, HP1) and fast atom bombardment. The spectra of the elastase digest fractions containing those modified heme-peptides show for:

<table>
<thead>
<tr>
<th>Heme-peptide</th>
<th>m/z</th>
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<tr>
<td>HP1</td>
<td>616.9</td>
<td>unmodified heme</td>
</tr>
<tr>
<td></td>
<td>734.9</td>
<td>heme + CCl₃</td>
</tr>
<tr>
<td></td>
<td>1349.3</td>
<td>heme + CCl₃ + C-D-K-L-H</td>
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<tr>
<td></td>
<td>1097.8</td>
<td>heme + CCl₃ + C-D-K</td>
</tr>
<tr>
<td>HP2</td>
<td>617.2</td>
<td>unmodified heme</td>
</tr>
<tr>
<td></td>
<td>698.4</td>
<td>heme + CCl₂</td>
</tr>
<tr>
<td></td>
<td>1311.5</td>
<td>heme + CCl₂ + C-D-K-L-H</td>
</tr>
<tr>
<td></td>
<td>1060.0</td>
<td>heme + CCl₂ + C-D-K</td>
</tr>
</tbody>
</table>

An extended elastase digest, 72 hours at 37°C, when checked by PDMS gave for HP1 a peak at m/z 1097.8 (figure 2), while HP2 gave a peak at m/z 1061.5. Both heme peptides were digested with aminopeptidase. Aliquots of the digests were screened by PDMS and HPLC every 24 hours. Completion of the reaction varies between 72 to 120 hours. The results of the aminopeptidase digest of HP1 shows a peak at m/z 855.2 which corresponds to the attachment of the modified heme containing three chlorines to cysteine.

734.9 + 1210 = 855.9

The results of the aminopeptidase digest of HP2 shows a peak at m/z 818.8 which corresponds to the attachment of the modified heme containing two chlorines to cysteine.

698.4 + 121.0 = 819.4

The results would appear to firmly establish the attachment of the prosthetic heme group to cysteine 93 of the hemoglobin β-chain (figure 3).
Identification of an N-7 conjugate of hepsulfam

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Hepsulfam (NSC 329660) is a bifunctional DNA alkylating agent presently in phase I clinical trials as a treatment for chronic myelogenous leukemia. Hepsulfam is active against CML in patients who have undergone blast crisis and resistant to the standard therapy using busulfan (1). Hepsulfam has a much broader spectrum of antineoplastic than busulfan (1). Hepsulfam has been shown to produce crosslinks in DNA (2) but the nature of these crosslinks is not known.

We have shown that hepsulfam reacts with the N-7 nitrogen of guanosine using a combination of mass spectrometry, reversed phase HPLC and ultraviolet spectrophotometry. We have also determined a DNA sequence motif sufficient for crosslinking of a small oligodeoxyribonucleotide.

The N-7 nitrogen of guanosine has been shown to be the target for many of the antineoplastic DNA alkylating agents currently used. Busulfan, the structural analog of hepsulfam, has been shown to produce interstrand crosslinks DNA by successive alkylation of the N-7 nitrogen atoms of guanylic acid residues (3). To determine if hepsulfam reacted analogously we studied the reaction of hepsulfam with guanosine in DMSO solution at 45 °C. A Kratos (Manchester, UK) Concept 1 H mass spectrometer was used to analyze the reaction mixture. Figure 1 shows the mass spectrum of this reaction mixture after three days incubation. The signals at 345, 477, 574, 760, and 857 amu correspond respectively to 7-(n-sulfamoylheptyl)guanine, 7-(n-sulfamoylheptyl)guanosine (Figure 2), 7-(n-sulfamoylheptyl)guanosine + sulfamic acid anion + proton, 1,7-Di(7-guanosinyl) heptane + sulfamic acid anion (Figure 3), 1,7-Di(7-guanyl) heptane + two sulfamic acid anions + proton. No signal was observed for the doubly charged 1,7-Di(7-guanosinyl) heptane (332 amu) or for 1,7-Di(7-guanyl) heptane + proton (399 amu).

To determine the position on the guanosine that is alkylated by hepsulfam we separated the reaction mixture by reversed phase HPLC. A large peak eluting at 22 minutes was the first to appear over the course of the reaction and thus was thought to be the adduct of hepsulfam to one guanosine. The peak was collected and concentrated and analyzed by UV spectrophotometry. Tong and Ludlam (3) have shown that the UV spectrum of N-7 alkylated guanosine undergoes an irreversible change at high pH corresponding to the opening of the alkylated ring where guanosine does not. To determine if the isolated HPLC fraction contains N-7 alkylated guanosine we observed changes in the UV spectra of guanosine, N-7 methyl guanosine, and the 22 minute HPLC peak isolated from the reaction mixture. Comparison of the pH dependence of the UV absorption spectra of N-7 methyl guanosine to that of the isolated HPLC fraction shows that the absorption band sharpens and shifts to a shorter wavelength, and that when the solutions are brought back to neutrality, the absorption does not return to the starting profile but remains relatively sharp. Examination of the shifts shown by guanosine show that the solution regains the original absorption profile after being taken to high pH. Thus we conclude that the 22 minute peak is indeed N-7 alkylated guanosine. Figure 4 shows the product of the base induced ring opening of N-7 hepsulfamoyl guanosine.

To determine the sequence DNA that hepsulfam crosslinks we synthesized two palindromic hexanucleotides, one with guanylic acid residues directly adjacent (5'-ATGCAT #5651) and one with guanylic acid residues separated by both adenylic and thymidylic acid residues (5'-GACGTC #5652). The oligonucleotides were reacted with hepsulfam and analyzed by RP-HPLC. The reaction mixtures were briefly heated to 60 C to denature the paired strands and an aliquot was immediately analyzed by RP-HPLC. When #5652 was analyzed without heating, a peak at 22 minutes indicative of the dimer and a peak at 29 minutes for the monomer were observed. When the starting material was analyzed after heating a single peak at 29 minutes was observed indicating nearly complete conversion to the monomer. In the solution containing both the oligonucleotide and hepsulfam 3 days of incubation peaks at both 22 and 29 minutes were observed after the solutions were denatured by heating. Thus we conclude that the undenaturable peak at 22 minutes observed in the drug treated solution is a crosslinked dimer. Oligonucleotide 5651 with adjacent guanylic acid residues showed no evidence of reaction.
DEVELOPMENT OF NEW METHODS FOR RAPID ANALYSIS OF DRUGS IN BIOLOGICAL MATRICES

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The determination of trace levels of drugs or other compounds in tissue and other physiological media requires analytical methods which are highly sensitive and yield confident compound identification. The goal of this work is to develop new approaches for the analysis of drugs and other organics in physiological samples which would be rapid, accurate and require a minimum quantity of sample. With respect to sample quantities, in the case of tissue samples, for example, it would be more desirable to sample a few milligrams of tissue obtained by needle puncture than harvest grams of tissue.

Previous work in our laboratory has demonstrated the ability to analyze picogram levels of drugs in one microliter aliquots of untreated urine in about three minutes total analysis time using thermal desorption directly into an ion trap mass spectrometer (ITMS). In a project for the Food and Drug Administration, we are attempting to extend this approach to other physiological media, such as milk and tissue. In this project, two anthelmintics, phenothiazine and piperazine, are being examined. Experimentally, the sample is thermally desorbed from a quartz wool bed directly into the ITMS with no chromatographic separation. The compounds are ionized using isobutane chemical ionization (CI), which selectively protonates these nitrogen-containing compounds to form (M+H)⁺ ions, greatly decreasing interferences from less basic compounds in the sample matrix. The (M+H)⁺ ions are isolated and collisionally dissociated to form characteristic fragment ions which confirm the presence of the targeted species. Initial studies with standards of phenothiazine, which yields a (M+H)⁺ ion with m/z 200 and is then dissociated to form ions with m/z 199 and 167, have suggested that detection limits of at least 50 parts per billion (50 pg in a 1 pL sample) are attainable. Piperazine, which yields a ((M+H)⁺ ion with m/z 87 with CAD ions with m/z 70 and 44, has yielded detection limits below 100 ppb in these initial experiments.

To assess whether samples could be analyzed with no sample preparation, milk and tissue homogenate were spiked with the drugs and analyzed directly by thermal desorption ITMS. Figure 1 shows the desorption profile for a 1 μL injection of a 5 μg/mL solution of piperazine in milk. Figure 2 shows a corresponding desorption profile for a 1 μL injection of a 500 ng/mL (500 ppb) solution of phenothiazine in milk. A study using beef tissue homogenate was also conducted with phenothiazine. An example of the resulting desorption profiles is shown in Figure 3, where 1 μL of tissue homogenate spiked with 10 ng/g of phenothiazine was examined. In these studies, no major interferences from the matrix were observed.

The required ppb (ng/g) detection limits for these drugs will require further development. In these initial studies, only one microliter aliquots of the solutions were used and larger aliquots could be injected to improve the detection limits. Also, a split open/split interface is used on the ITMS transfer line which allows only a few percent of the effluent to enter the ITMS. Adjusting this ratio might also improve the achievable detection limit.

In cases where sample preparation will be required for concentration of the targeted compounds to reach the specified detection limits or elimination of matrix interferences, methods based on countercurrent dialysis are being developed. Dialysis capillaries are used to separate the higher molecular weight materials in the samples, such as cell debris, proteinaceous material and lipids, from the smaller molecular weight compounds, which are deposited on a sorbent trap for subsequent thermal desorption. Initial experiments with this approach have shown that good isolation can be achieved in a few minutes with small quantities of samples.

In conclusion, these initial studies have shown that thermal desorption ITMS has great potential for the rapid determination of targeted drugs in milk and meat. Additional work is being conducted to extend the ultimate detection limits to the low ppb levels using small quantities of samples, including modifications in the thermal desorption device and transfer line into the ITMS, as well as development of rapid countercurrent dialysis methods to concentrate the drugs on a sorbent bed for subsequent analysis.

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The 39th ASMS Conference on Mass Spectrometry and Allied Topics


Figure 1
Piperazine In Milk
Direct Thermal Desorption ITMS
MS/MS of Mass 87

1 uL Injection
Conc. 5 ug/mL

m/z 44
Area 1378

m/z 70
Area 230

Scan # 468 469 470 471 472 473 474 475 476 477 478 479 480
Time (minutes)

Figure 2
Phenothiazine in Milk
Direct Thermal Desorption ITMS

MS/MS of Mass 200

1 uL Injection
Conc. 500 ng/mL

m/z 167
Area 284
Area 228

m/z 199
Area 4352
Area 3953

Scan # 268 269 270 271 272 273 274 275 276 277 278
Time (minutes)

Figure 3
Phenothiazine in Beef Homogenate
Direct Thermal Desorption ITMS

MS/MS of Mass 200

1 uL Injection
Conc. 10 ug/g

m/z 167
Area 2500

m/z 199
Area 17000

Scan # 258 259 260 261 262 263 264 265 266 267 268 269 270
Time (minutes)
RAPID SCREENING TECHNIQUES FOR TAXOL AND OTHER TAXANES USING DCI-MS/MS.

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West Lafayette, Indiana 47907 USA

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Purdue University, W. Lafayette, Indiana 47907 USA

Taxol, a novel diterpene originally isolated from the bark of the western yew, Taxus brevifolia, is a potent antileukemic agent. It shows excellent activity in screens against many types of cancer, and has a unique mechanism of action as the only plant product known to induce assembly of tubulin into microtubules. These extremely promising characteristics of taxol as an effective anticancer drug are offset by the difficulty of synthesizing taxol and its natural shortage (30 pounds of bark to isolate 1 gram), which makes it critical to develop highly sensitive screening techniques to identify taxol in crude extracts. Alternative sources of taxol and related taxanes should also be screened. We use positive and negative desorption chemical ionization tandem mass spectrometry (DCI-MS/MS) to identify and approximately quantitate taxol in plant material, especially plant extracts, so as to direct isolation efforts towards the most appropriate samples. Tandem mass spectrometry combines separation power with capabilities for compound identification; this allows targeted compounds to be recognized in complex mixtures.

Mass spectrometric experiments were performed using a Finnigan TSQ-700 triple-stage quadrupole mass spectrometer. Samples were dissolved in either methanol or ethanol and ca. 1 µl aliquots were introduced on the rhenium wire DEP tip. The probe was then inserted into the chemical ionization source (ammonia, 0.5 torr) and rapidly heated (0-600°C at 100°C/s). Collision activated dissociation product-ion, parent and neutral loss spectra were acquired by leaking argon into the second quadrupole collision region (1.8 mtorr) and accelerating the ions to 20-40 eV (lab).

Taxol shows both an (M+H)+ ion, m/z 854, and an (M+NH4)+ ion, m/z 871, under positive ammonia desorption ionization conditions. The compound fragments in a structurally characteristic fashion (Fig. 1a) and the MS/MS product-ion spectrum of the (M+H)+ ion is also structurally diagnostic (Fig. 1b). When 10 micrograms of Taxus baccata bark extract was examined by DCI, it gave the featureless mass spectrum shown in Fig 1c. By contrast, the same amount of sample gave the MS/MS product-ion spectrum shown in Fig 1d.

Searches for taxol analogs which contain the biologically active side chain are made using selected parent and neutral loss scans. For example, analogs with modifications in the ester side chain were sought using negative chemical ionization and parent spectra of m/z 526, the ion corresponding to loss of the side chain in taxol, m/z 853. Candidate ions are then characterized by their conventional MS/MS spectra. Figure 2 displays a negative ion parent spectrum of m/z 525 acquired from the crude extract of T. baccata which shows several analogs of taxol are present. MS/MS product-ion spectra have identified m/z 831 as cephalomannine. In addition to searching for analogs with modifications in the ester side chain, analogs which contain the intact side chain are present. In this experiment (positive DCI), a constant neutral loss of 285 was chosen in order to seek analogs which have the intact C-13 side chain but differ elsewhere in the molecule. This spectrum of the same crude extract is reproduced as Fig 3. In addition to protonated taxol at m/z 854 and its NH4 adduct, at m/z 871, signals are seen at m/z 836, potentially a dehydrated analog, m/z 882, potentially an ethylated analog, and m/z 896, potentially the acetylated taxol and m/z 802, an unassigned taxane.

Using this methodology taxol has been identified and quantitated using standard addition in plant cell tissue cultures at the ppm level and taxol and several analogs have been identified in crude extracts from T. baccata and T. brevifolia bark. These studies demonstrate the benefits of the speed and low detection limits of tandem mass spectrometry and its applicability to complex mixtures.
Figure 1. DCI mass spectra from (a) pure taxol and (c) crude bark extract. MS/MS product-ion spectra of m/z 854 from (b) pure taxol and (d) bark extract.

Figure 2. Negative ion MS/MS parent scan of m/z 525 from a crude extract of T. baccata bark.

Figure 3. MS/MS neutral loss scan of 285 from a crude extract of T. baccata bark.
Class Characteristic Fragmentation of Glutathiones Under
High Energy Collisionally Induced Decomposition
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Department of Chemistry and Biochemistry,
University of Maryland Baltimore County, Baltimore, MD 21228

Both the enzymatically catalyzed and the chemical reactions of glutathione are important parts of the defense system of every cell against xenobiotic insult. Such conjugation often inactivates xenobiotic agents, and also causes significant changes in the activities of endogenous substrates.

Mass spectral analysis of this important and widely encountered family of metabolites was not satisfactorily achieved before the advent of desorption techniques. In cation spectra, abundant protonated and/or natriated molecular ions are usually observed, along with class characteristic ions of 306 or 308, comprising glutathione. Other fragmentation is usually minimal.

Recently various kinds of tandem mass spectrometry have been evaluated for the dual purpose of working with incompletely purified metabolites and of inducing fragmentation by collisional activation. Indeed, under collision activation more fragmentation is observed. Because of the strategic value of class characteristic ions, most investigators have proposed candidates, sometimes on the basis of a single glutathione sample. Thus Straub proposed the loss of 275 amu as class characteristic decomposition under his 29 volt collision; Pearson et al. proposed the loss of 129 under 0-100 ev collisions; Heeremans et al. recommended the loss of 146 amu under 45 ev collisions; Haroldson et al., analyzing low energy collisions of seven different conjugates, observed all three of these losses, however none in all seven spectra. Although the latter group favored 129 for constant neutral loss strategies, their report indicates that \([M+H]^+ - 232\) ions were observed in the daughter ion scans of all seven samples.

At least three papers in the literature report high energy collisionally induced decomposition of protonated molecular ions from glutathione conjugates on multisector instruments. Lay et al. reports the elimination of neutral molecules weighing 275 and 129 on a triple sector EBE instrument. In a study by Stock et al. using a four sector instrument of BEEB configuration, losses of 129 and 175 are observed, however the spectra were not recorded in low enough range to see loss of 275. Nelson et al have reported the losses of 75, 130 and 274 amu neutrals in two sector linked scans of a molecular ion radical formed by field desorption.

All mass spectrometric measurements in this study were obtained using a JEOL (Tokyo, Japan) HX110/HX110, four sector mass spectrometer with geometry of EEBE. The FAB gun was operated at 6 kV and the FAB gas was xenon. Tandem measurements were obtained by utilizing all four sectors (EBEB) on the instrument. In these experiments, helium was used as a collision gas at pressures sufficient to attenuate the precursor ion by 80%. The accelerating voltage was 10 kV, and the collision cell was floated at 4 kV. Resolution in both MS-1 and MS-2 was 1000. For both scanning modes, the compounds were dissolved in methanol (Fisher Scientific Co., Fairlawn, NJ) and the matrix was either thiglycerol (Sigma Chemical Co., St. Louis, MO) or 3-nitrobenzyl alcohol (Aldrich Chemical Co., Milwaukee, WI).

Fifteen glutathione conjugates were analyzed, including both mono and di-conjugates. The glutathione conjugates encompassed a wide range of compounds. All of the conjugates showed fragmentation resulting from the cleavage of the sulfur bond with the charge being retained either on the "R" group or the glutathione moiety. In addition to sulfur cleavages the vast majority of the conjugates showed fragmentation.
resultant from cleavages within the tripeptide. Cleavages at the γ-glutamyl carbonyl and at the amide bond of glycine were prevalent. The monoconjugate of dimethylbilirubin fragmented in a slightly different manner. Due to the fragility of the methylene bridge, the fragmentation was dominated by "R" group fragmentation. Furthermore, due to the basicity of the "R" group the charge was preferentially retained on the "R" group. None the less, this glutathione conjugate still exhibited class characteristic sulfur bond cleavage fragmentation.

Table 1. Fragmentation of Glutathione Conjugates with High Energy CID

<table>
<thead>
<tr>
<th>Parent Compound</th>
<th>[M+H]⁺</th>
<th>Fragmentation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>mono-conjugates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cyclophosphamide</td>
<td>532</td>
<td>a,b,e,f,i,k,d,R</td>
</tr>
<tr>
<td>di-thiolic phosphoramide</td>
<td>458</td>
<td>a,b,f,i,k,R</td>
</tr>
<tr>
<td>di-thiolic hemiacetal</td>
<td>458</td>
<td>a,b,f,i,k,R</td>
</tr>
<tr>
<td>dinitrobenzene</td>
<td>474</td>
<td>a,b,e,f,i,k,d,R</td>
</tr>
<tr>
<td>styrene oxide</td>
<td>428</td>
<td>a,b,e,f,i,k,d,R</td>
</tr>
<tr>
<td>hexyl</td>
<td>392</td>
<td>a,b,e,f,i,k,R</td>
</tr>
<tr>
<td>lactoyl</td>
<td>394</td>
<td>a,b,e,f,i,k,d,R</td>
</tr>
<tr>
<td>lactoyl methyl ester</td>
<td>408</td>
<td>a,b,e,f,i,R</td>
</tr>
<tr>
<td>lactoyl ethyl ester</td>
<td>936</td>
<td>d,R</td>
</tr>
<tr>
<td>dimethylbilirubin</td>
<td>936</td>
<td>a,b,e,f,i,R</td>
</tr>
</tbody>
</table>

| di-conjugates              |        |                |
| phosphoramidemustard        | 763    | b,f,i          |
| di-aziradine quinone        | 936    | a,b,e,f,i,d,R  |
| di-aziradinequinone         | 979    | a,b,e,f,i,d,R  |

* see scheme below for fragmentation designation

![Diagram of glutathione conjugate fragmentation](image)

Partial Characterization of Urinary Metabolites of U-78,875 in Monkey Urine by Tandem Mass Spectrometry

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Drug metabolite identification in humans and animals is of importance from both a scientific and product development perspective. The determination of drug metabolite structures has traditionally occurred after a compound has been selected for extensive preclinical toxicological evaluation since drug metabolism studies are expensive, relatively time consuming and, to be comprehensive, require the synthesis of a radioisotope labeled drug. It has been suggested that it is possible to characterize or partially characterize many drug metabolite structures relatively rapidly using "MS-MS" screening techniques and tandem quadrupole mass spectrometry. Here we report the rapid identification of metabolites of a novel anxiolytic drug candidate by tandem mass spectrometry.

U-78,875 (3-(5-cyclopropyl-1,2,4-oxadiazol-3-yl)-5-(1-methylbutyl)-imidazo[1,5-a]quinoxalin-4(5H)-one) is under development as a non-benzodiazepine anxiolytic and is presently in Phase I clinical evaluation. The purpose of the present study was to identify metabolites of U-78,875 present in monkey urine by LSIMS ionization and tandem quadrupole mass spectrometry. This was to be accomplished by obtaining high resolution electron ionization data for U-78,875, CAD spectra of U-78,875 and structural analogues and by the judicious use of parent and/or neutral loss scans suggested by these data.

Methods: ODS extracted urine was dissolved in water and chromatographed on ODS using gradient elution reversed phase HPLC. Fractions were collected every 5% change in solvent composition. High resolution El mass spectral data were recorded using a VG ZAB-2F high resolution mass spectrometer and direct probe electron ionization. Low resolution collisionally activated dissociation (CAD) mass spectra were obtained using a Finnigan MAT TSQ-70 tandem quadrupole mass spectrometer. Particle beam data were obtained using an Extrel designed interface and a Finnigan 4500.

Results: High resolution El analysis of U-78,875 and low-resolution CAD analysis of U-78,875 and structural analogues indicated that the only useful scan function for "screening" for metabolites using tandem mass spectrometry were parents of m/z 69 (cyclopropylcarbonyl derived from the substituted oxadiazole ring, Figure 1. Neutral loss of 68 daltons from MH⁺ was also observed but found to be far less sensitive in detecting metabolites). Several parent ions of m/z 69 were observed in drug related samples that were not observed in the control samples. Positive daughter ion mass spectral data obtained for parent ions m/z 336, 295, 352, 310, 433, 390, and 528 indicated the presence of U-78,875 and N-deisopropyl U-78,875 and partially characterized aryl hydroxyl, aryl hydroxyl-N-dealkyl, aryl glucuronide, aryl glucuronide-N-dealkyl, aryl O-sulfate and aryl O-sulfate-N-dealkyl metabolites. From these data it was concluded that metabolic pathways involved in the biotransformation of U-78,875 include N-dealkylation, aryl hydroxylation and subsequent sulfation or glucuronidation of hydroxy metabolites. Balance data was not obtained by this method. These data clearly demonstrated both the 1) utility and the 2) limitations of tandem quadrupole mass spectrometry in characterizing important metabolic transformations of a drug prior to the synthesis of radioisotope labeled drug, in that 1) rapid metabolism information was obtained, however, 2) a number of metabolites (characterized at a later date using radioisotopes) were not detected using this methodology.
In a related study, metabolites of U-78875 were detected and characterized by particle beam LC-MS. This technique was able to characterize U-78875, N-dealkyl metabolites, hydroxy metabolites, and the highly polar glucuronide conjugates. These and other data strongly suggested that the design of PB-MS used in these studies has utility in the structural characterization of highly polar drug metabolites, especially if a heated target is used.

Figure 2: Observed metabolic transformations of U-78875 in the rat.

Characterization of Stereoselective Benzylic Glucuronidation of the Antipsychotic Agent BMY-14802 Using Mass Spectrometry

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Introduction
BMY-14802 (±)-(±)-1-(4-(4-fluorophenyl)-4-hydroxy-1-butyl)-4-(5-fluoro-2-pyrimidinyl) piperazine hydrochloride is currently undergoing evaluation as a new antipsychotic agent. In vitro studies using rat liver microsomes have established two potential routes of metabolism: cytochrome P-450-mediated N-dealkylation and oxidation, thought to be mediated by alcohol dehydrogenase. N-dealkylation was a major route of metabolism seen previously with structurally related drugs buspirone and tiospironem vivo (1) and in vitro (2). The present study was initiated in order to determine whether these two routes of metabolism could be observed in human subjects. The presence of a free hydroxyl group in the molecule suggested that conjugation could also occur. Urine from patients dosed with BMY-14802 was analyzed by HPLC before and after hydrolysis with β-glucuronidase/sulfatase derived from helix pomatia. This enzyme was previously demonstrated to efficiently hydrolyze urinary conjugates of buspirone (1).

Results and Discussion
Urine from a subject who had been dosed with 400 mg of BMY-14802 was extracted using a C18 solid-phase extraction column. The extract was analyzed by a reversed-phase HPLC system on a C18 column using methanol/0.1 M aqueous ammonium acetate/ triethylamine gradient. A small amount of BMY-14802 was observed (retention time 36 min). There was a large peak at a retention time of 26 min which appeared to consist of two components. This peak did not correspond in retention time to any of the known analogs or metabolites of BMY-14802. The urine was hydrolyzed with helix pomatia β-glucuronidase/arylsulfatase and the products were isolated using a C18 extraction column. In the 2-4 hr post-dose urine it was evident that hydrolysis had reduced the amount of the polar metabolite observed previously and that it now appeared to consist of a single component. There was a concomitant sizeable increase in a peak with a retention time corresponding to authentic BMY-14802. If the peak remaining with a retention time of 26 min was also conjugate, it was not clear why the de-conjugation had failed to go to completion.

BMY-14802 had been administered to the human subjects as a racemic mixture. This raised the possibility that the polar conjugates were glucuronide diastereomers and that one of them was a poor substrate for the helix pomatia β-glucuronidase. If both polar metabolites were indeed glucuronides, it was reasoned that it should be possible to methylate the carboxylic acids on the sugar moiety. To test this hypothesis, the urinary metabolites were dissolved in aqueous methanol and treated with ethereal diazomethane. HPLC analysis showed that diazomethane treatment increased their retention times from 26 mins to 33 mins. This confirmed that the conjugates contained free carboxyl groups. Interestingly, the HPLC characteristics of the methyl ester derivatives improved considerably when compared with the free acids so that they could be readily separated into components A and B using an isocratic system. The urine was hydrolyzed with β-glucuronidase, extracted and treated with diazomethane. HPLC analysis revealed that peak A was lost on hydrolysis and that peak B was unaffected. The two methylated derivatives A and B were isolated from the urine, purified by preparative HPLC and analyzed by DCI/MS (Figure). A protonated molecular ion appeared at m/z 421 in both methylated metabolites and significant fragment ions were observed at m/z 519 (MH - F), 347 (MH - glucuronide) and 331 (MH - O-glucuronide). Saponification of the individual methylated glucuronides and analysis by FAB/MS provided confirmation of the metabolite structures as BMY-14802 glucuronide diastereomers. The configuration of the diastereomers was assigned from biosynthetic studies with individual BMY-14802 enantiomers and UDPGA-fortified human liver microsomes. (+)-BMY-14802-glucuronide methyl ester
corresponded to compound A and (-)-BMY-14802-glucuronide methyl ester corresponded to compound B. (-)-BMY-14802 was a better substrate for human glucuronosyl transferase than (+)-BMY-14802. This was due to a lower apparent \( K_m \). Interestingly, there was an even greater difference in the apparent \( K_m \)s for the (-) and (+)-enantiomers with bovine liver glucuronosyl transferase. Paradoxically, (-)-BMY-14802-glucuronide was a very poor substrate for \( \beta \)-glucuronidases whereas (+)-BMY-14802-glucuronide was a very good substrate. This provides evidence to suggest that caution should be exercised when quantitative estimates of metabolite excretion are obtained after de-conjugation of human urine with \( \beta \)-glucuronidases. Supported by NIH grant ES 00267 and Bristol-Myers Squibb.

References


Figure: (a) DCI mass spectrum of (+)-BMY-14802-glucuronide methyl ester (component A). (b) DCI mass spectrum of (-)-BMY-14802-glucuronide methyl ester (component B).
SENSITIVE AND SPECIFIC ANALYSIS OF HEXOBARBITAL ENANTIOMERS AND THEIR METABOLITES BY GC/ELECTRON CAPTURE NICI MS

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Hexobarbital (HB), a sedative/hypnotic and anesthetic drug, is used as a model substrate for the assessment of changes in the oxidative activity of hepatic CYP in human subjects and in animals (1). It is metabolized by alicyclic oxidation to 3'-hydroxyhexobarbital (3'-OH-HB), which is then oxidized to the 3'-keto metabolite (3'-keto-HB). On the other hand, epoxidation of the 1',2'-olefinic bond ultimately produces 1,5-dimethylbarbituric acid (1,5-DMB), and there are additional minor routes of metabolism, including N-demethylation. HB is a chiral drug and its metabolism is polymorphic, cosegregating with the stereoselective polymorphic oxidation of mephenytoin. This raised the possibility that there may be subtle difference in the stereoselective metabolism of HB by PMs, IMs, and EMs of mephenytoin. In order to investigate this possibility, an assay was required that could simultaneously monitor the individual HB enantiomers together with their metabolites. We have developed a highly sensitive and specific assay, based on gas chromatography/electron capture negative ion chemical ionization mass spectrometry (GC/NICI MS), for the analysis of HB enantiomers and their metabolites in plasma and urine as pentafluorobenzyl (PFB) derivatives. The PFB derivatives of HB enantiomers and their metabolites had excellent GC properties except for 3-OH-HB-PFB which gave a rather broad peak, however, conversion to the TMS derivative markedly improved its GC characteristics. All of the derivatives were readily separated by capillary GC (Fig. 1). Intense NICI spectra were observed for the HB derivatives. One major ion corresponding to [M-PFB]$^-$ was observed for each derivative except for 1,5-DMB where M$^+$ was observed. S-(+)-[5-2H3]-HB and R-(−)-[5-2H3]-HB were synthesized for clinical studies along with (±)-[1,5,5-2H3]-hexobarbital. Deterated analogs of the major metabolites were also synthesized for use as internal and reference standards. NICI spectra of S-(+)-[4-2H6]-HB-PFB, R-(−)-[4-2H6]-HB-PFB and [4-2H6]-HB-PFB showed intense ions at m/z 235, m/z 238 and m/z 241 respectively (Fig. 2).

The availability of all the relevant deuterated standards and a highly sensitive and specific method of detection allowed the quantification of HB enantiomers and their major metabolites in biological fluids to be carried out after administration of a small oral dose of HB. Four internal standards were employed in the assay: [4-2H6]-HB was used as a standard for HB and N-desmethyl-HB, [4-2H6]-3'-keto-HB for 3'-keto-HB, [6-2H6]-3'-OH-HB for 3-OH-HB and [6-2H6]-1,5-DMB for 1,5-DMB. The lowest detection limits were 50 ng/ml for 3-OH-HB and 1.0 ng/ml for the other compounds (signal to noise ratio, 5:1). Standard curves were linear between 1.0 ng/ml and 10 μg/ml; linearity beyond these limits was not tested because they were considered out of range for likely clinical doses. Recovery of HB and metabolites ranged from 25-65 % and intra- and inter-day variability was in the range 7.7 % to 17.6 %. The assay was applied to the analysis of HB and metabolites in the plasma and urine of a subject given a 100 mg oral dose of pseudoracemic HB containing equal amounts of S-(+)-[4-2H6]-HB and R-(−)-[4-2H6]-HB. This preliminary investigation confirmed the stereoselective disposition of HB in humans as reported previously. The two enantiomers demonstrated very rapid absorption which was completed within the first half hour after administration. (-)-HB was eliminated much more rapidly from the plasma than (+)-HB resulting in higher plasma concentrations of (+)-HB over time. This difference in elimination also resulted in significantly higher plasma concentrations of the 3'-keto and DMB metabolites from the (-)-enantiomer. Only low plasma levels of the N-desmethyl metabolite were detected while the 3'-OH metabolite was present only in trace to unmeasurable amounts from both enantiomers. Pharmacokinetic parameters from these data revealed that R-(−)-HB had a tenfold greater oral clearance than S-(+)-HB (147.5 versus 16.8 L/hr) and a threefold shorter terminal half life (0.93 versus 3.11 hr). Urinary excretion data showed 1,5-DMB as the major urinary metabolite of both enantiomers. More of the (-)-HB was excreted as the 3'-OH, 3'-keto and DMB metabolites than (+)-HB, while the reverse was the case for unchanged drug and N-desmethyl-HB.

Supported by U.S. Public Health Service grants GM-31304, ES-00267 and RR00995.

Fig. 1: Reconstructed total ion current chromatogram of HB and its putative metabolites (a) 1,5-DMB-PFB (b) HB-PFB (c) 6'-keto-HB-PFB (d) 3'-OH-HB-TMS-PFB (e) 3'-keto-HB-PFB (f) N-desmethyl-HB-bis-PFB.

Fig. 2: NICI spectra of (a) S-(+)-[2H0]-HB-PFB, (b) R-(−)-[2H3]-HB-PFB and (c) (±)-[2H6]-HB-PFB.
ORGANIC ION IMAGING
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Secondary ion mass spectrometry offers the possibility of determining the spatial distribution of selected compounds in biological tissue. We have developed an ion microprobe to perform this type of analysis. The instrument consists of a focused Cs* ion gun, sample stage, secondary ion source, quadrupole mass analyzer and detector with a 80286-based computer system to control the components, collect data, and display image data in real time. Distributions are determined by rastering the Cs* beam across the target, while monitoring the intensity of selected ion(s) at each raster position. Distributions are displayed as color images (using a modified thermal scale) and cartesian plots of intensity vs. position in either X or Y coordinates.

In this paper, we report on the characteristics of the instrument using samples that can be readily identified by other methods. Historically, grids of one form or another have been used to establish image fidelity and spatial resolution of different microprobes. Figure 1 is the secondary ion image observed from a hexagonal grid with 75 micron wire diameter and 2 mm spacing between parallel wires. From this image, it is apparent that the spatial resolution of the instrument is about 75 microns. Specifically, the apparent ratio of spacing-to-wire diameter (16.7), to the known ratio (26.7), indicates that the wire appears to be approximately twice its actual width. The hexagonal shape of the grid attests to the fidelity of the image system. The cartesian plot at the bottom of Figure 1 is a quantitative display of the ion intensity along the horizontal cursor line. Note that the color micrograph of Figure 1 suffers in the translation to grey shades in reproduction. This is evident from the cartesian plot in the lower portion of Figure 1.

Figure 2 shows the m/z 170 image taken from a mushroom that had been doped with an ethanol solution of methylphenylpyridinium (MPP*) iodide; in the image, black represents the highest intensity and shades of gray are used to represent relative intensity. When a drop of the ethanolic solution was deposited onto the mushroom slice, most of the solution ran to the sides of the sample stage, where the solution was subsequently absorbed by the mushroom. Consistent with this, the intensity of m/z 170 is greatest at the sample periphery. The dark right angle near the top of the image shows the effects of the knife that was used to slice and quarter the mushroom. The field of view of the microprobe is about 1.5 centimeters, which is approximately the size of the sample shown in Figure 2. Most ion microprobes have fields of view and maximum image size of 0.5 mm. or less, consistent with the transmission requirements of quadrupole and sector mass analyzer. For the microprobe described here, the secondary ion optics are rastered with the primary ion beam to permit a much larger field of view. To prove this point, a special, moveable electron impact ion source was built to mimick secondary ion emission. With the Cs* gun off, the secondary ion optics were rastered across the exit of the electron impact source. The image of acetone molecular ions emitted from the source is shown in Figure 3. The profile of the beam clearly shows a resolution element of approximately 100 microns. This resolution element reflects the field of view of the secondary ion source/quadrupole assembly at any particular spot on the sample. Were the field of view larger, the cartesian plot in the lower portion of Figure 3 would show a broader peak.

Acknowledgment
Research sponsored by the National Institute of General Medical Sciences, under Interagency Agreement GM-41617 and by the U. S. Department of Energy, Office of Basic Energy Sciences, under contract DE-AC05-82OR21400 with Martin Marietta Energy Systems, Inc. Casey C. Grimm gratefully acknowledges a postdoctoral fellowship sponsored by Oak Ridge Associated Universities.
Figure 1. (Upper Left) Image of hexagonal grid. Cartesian profile below the image corresponds to intensity along the horizontal cursor.

Figure 2. (Left) MPP* image from doped slice of mushroom. Dark portions at the periphery reflect absorption of ethanolic MPP* from target stage.

Figure 3. (Above) Acetone molecular ion image obtained by rastering the secondary ion source optics across electron impact source. Cartesian profile shows the limited field of view of the secondary source at any point.
Improved Sensitivity for FAB-MS on Magnetic Sector Instruments Using Pulsed Bombardment and Array Detection

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It has long been recognized that the ionization events immediately following the initiation of pulsed bombardment may be quite different from subsequent events. This observation may be noted by using a number of instrumental designs and configurations. With regard to our studies a particularly important observation is that for certain preparations, the intensity ratio of sample to matrix ions is significantly enhanced in the initial stages of bombardment. We have used an array detector fitted to a double-focusing mass spectrometer to study this phenomenon.

Our equipment lacked the circuitry to synchronize atom bombardment pulses with the collection of data by the array detector. To simulate the effect of pulsed atom bombardment, samples were prepared on one side of a double-sided FAB probe which allowed the sample to be manually rotated in and out of a stable 6 keV xenon atom beam. The array detector was set to detect and log data at regular intervals. The detection cycle time with that equipment was typically in the range 65-100 ms.

Figure 1 shows the molecular region mass spectra obtained at 100 ms intervals from 400 attomoles of the peptide eldeoisin (sequence pyro-EPSKDAFGLM-NH$_2$) in glycerol. Differential ionization of the protonated molecular signal at m/z 1188 and the glycerol matrix cluster ion at m/z 1197 are observed with the peptide signal diminishing and the glycerol cluster increasing in intensity as bombardment ensues. The quantity of peptide required to produce the signal/chemical noise ratio observed for the protonated molecule in these spectra represents an enhancement of better than four orders of magnitude over the quantity required to yield similar enhancements for all classes of molecules that we have examined that show the pulsing effect upon the initiation of bombardment.

Sample preparation is the key factor in optimizing for pulsed FAB. In contrast to sample preparations in glycerol, very few samples exhibit any pulsing behavior when prepared in nitrobenzene alcohol (NBA) matrix. While NBA is often superior as a matrix for conventional FAB, glycerol is usually superior for pulsed FAB. We attribute this observation to the higher surface tension of glycerol compared to NBA, and the formation of surface layers of analyte that are removed in the first few hundred milliseconds of particle bombardment.

In our studies, we have noted that the ions observed immediately following the initiation of bombardment may be of a different nature to those evolved subsequently. For example, the FAB mass spectrum of Prostaglandin A$_1$, obtained under steady bombardment conditions shows a signal corresponding to the ion [M+H-H$_2$O]$^+$ and no significant [M+H]$^+$ ion$^3$. Figure 2 shows three exposures of a sample of Prostaglandin A$_1$ in glycerol. The relative intensities of the protonated molecule and the water loss signals are quite different in the three exposures. These data were recorded for equal durations and all masses in the observed mass range are recorded simultaneously. This suggests that the sample was rotated into the atom beam at differing points in the integration window. Therefore the data actually reveal the effects of phenomena of shorter duration than the 65 ms cycle time of the array detector. This in turn suggests that differential ionization events occur on a time scale faster than we can presently detect.
Figure 1. Molecular ion region of eledoisin obtained for 400 attomoles of eledoisin in glycerol. Successive mass spectra were obtained at 100 ms intervals. These mass spectra illustrate the differential evolution of the analyte species and the matrix chemical background. The differentiation between analyte and chemical background, coupled with array detection, leads to improved sensitivity.

References:
In 1987 we proposed a new instrument incorporating wide angle multichannel array focal plane detection with high field high resolution capability for ultrasensitive high mass analysis of Biopolymers by FAB and FD. The concurrent requirements of Gross et al. (Nebraska) for four sector tandem MS with low noise systems for post-translational modification in proteins. A two sector version of the ZAB, the ZAB-2SE FPD, has now been constructed incorporating one wide angle (1.5:1) array for high sensitivity, low resolution survey analysis up to 15 kilodaltons, and the option of a second array (1.05:1) for narrow angle (5X window) high resolution analysis. The instrument also retains the off-axis multiplier detection of the ZAB 2SE together with its Linked Scan, CAD sequencing capabilities.

The basic specifications we proposed in 1987 are summarised in Figure 1, and these include the foreseeable requirements for biopolymer MS analysis of unknown structures, with respect to mass accuracy, mass range and sensitivity, for the next 5-10 years.

The instrument is in the final stages of construction, and in this paper we present preliminary data which illustrate the basic capabilities which will be routinely available. Refinements in data collection and in hardware and software related to mass calibration and assignment are still in progress, and therefore the mass scale and mass assignments in the figures shown, serve only as a guide at this stage of construction.

The significant increase in sensitivity of analysis of high mass (2-14kDa) biopolymers from the combined use of high field magnet large geometry instruments with focal plane array detection is expected to be achievable only with the use of low chemical noise ionisation systems (see later). Nevertheless, using conventional FAB ionisation without low chemical noise modifications (e.g. continuous flow FAB) one expects to achieve equivalent sensitivity of detection to that obtained scanning across the point detector, and over shorter ionisation times. This is illustrated with the array detector spectrum of 1 picomole of insulin in Fig 2 illustrating a 2 x 1 second picture of simultaneous detection over the 4,500 to 6,900 mass range.

The spectrum is background subtracted using an acquisition of matrix alone to remove some of the chemical noise derived artefacts, and it compares favourably in quality with the 1 picomole point detector spectrum obtained by summing and averaging over 20 seconds, followed by peak detection and smoothing. The ability to obtain the required equivalent data in a 1 second timescale, with the majority of sample still remaining can clearly be exploited. The major advantage of the wide angle array is of course expected to be seen with low chemical noise ionisation systems such as F.D. We introduced the use of high field F.D. analysis in particular in the glycoprotein/carbohydrate field some 15 years ago in the study of post-translational modification in proteins. A two sector version of the ZAB, the ZAB-2SE FPD, has now been constructed incorporating one wide angle (1.5:1) array for high sensitivity, low resolution survey analysis up to 15 kilodaltons, and the option of a second array (1.05:1) for narrow angle (5X window) high resolution analysis. The instrument also retains the off-axis multiplier detection of the ZAB 2SE together with its Linked Scan, CAD sequencing capabilities.

The spectrum shown in Fig 3, labelled as "No Dilution", is estimated as 5 picomoles from carbohydrate analysis. Dilutions of 1 in 5 and 1 in 50 confirmed the minimum level of point detection. Figures 4 and 5 illustrate the production of clear strong data on the array detector for the F.D. experiment on 1 in 5 and 1 in 50 dilutions respectively, showing summed pictures over the desorption profile from 25 to 35 milliamps. Interestingly, a single 1 second shot shown in Figure 6 for the 1 in 100 dilution is again of recognisable quality to the summed picture in Fig 5. Figures 7 and 8 illustrate the array spectrum of the F.D. of a 1 in 100 dilution of the sample, showing better quality data both for summed and individual pictures than the "no dilution" point experiment.

These data illustrate the potential of the new instrument for wide mass range high sensitivity detection applied to real problems in the field of biochemistry, biotechnology, and molecular biology, allowing increased sensitivity up to a factor of 100. This takes analysis of carbohydrates and peptides into the low femtomole region when low chemical noise ionisation systems are used, allowing retention of the majority of sample for other purposes even where high chemical noise ionisation is used. The mass accuracy specifications together with testing of the high resolution (10,000 R.P.) array at the double focussing...
position, are expected to be achieved within the next few months.


The authors thank the Wellcome Trust and Medical Research Council for financial support.

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- FOCAL PLANE DETECTION WITH MAXIMUM WIDE ANGLE ARRAY
- SURVEY SPECTRUM: 1,000 R.P. MASS ACCURACY 0.3 Da AT 8 KDa
- 10 PENTAMOLE DETECTION CAPABILITY
- 8 KDa >3:1 S/N
- 15 KILOGRAM MASS RANGE
- HIGH FIELD MAGNET
- HIGH RESOLUTION ARRAY
- 10,000 R.P. MASS ACCURACY 0.12 Da
- POST ACCELERATION
- PRESERVE POINT DETECTOR CAPABILITY

The authors thank the Wellcome Trust and Medical Research Council for financial support.
OPTIMIZATION OF MICROCHANNEL PLATE ARRAY DETECTORS USING ONE-DIMENSIONAL PHOTODIODE ARRAY READOUT

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Microchannel plates (MCPs) are a popular component of many spectrometers, enough so that their performance has been extensively reported in the literature\(^1\)\(^2\). Common configurations use a single plate, or two in tandem, with a one-dimensional readout system such as a photodiode array (PDA).

Despite this research, the behavior of many MCP configurations have not been explored, mainly due to the number of design factors. What possible improvements in resolution and gain can we achieve with some of these untested devices? How critical is MCP performance to overall spectrometer performance?

To avoid testing a prohibitively large number of detector configurations, we turned to statistical experimental design. This permitted us to predict MCP detector behavior using a smaller sample size. Experimental design allowed us to determine the relative importance of each factor studied, and to make a preliminary choice of the configuration providing highest gain and/or spatial resolution.

Resolution is determined by the size and compactness of the charge cloud exiting from the MCP, and is affected by many of the same parameters which influence MCP gain. We chose to study the following factors: MCP pore size, separation between the MCP output and the phosphor screen (thus the electric field in that region), bias angle of the MCP pores relative to the detector axis, a proprietary Galileo image enhancement process, and the length to diameter (L/D) ratio of the pores. Note that even with only two levels per factor, the experimental space has \(2^5 = 32\) possible configurations; using a fractional factorial design we covered the same experimental space with only eight trials. The effect of bias voltage across the plates is well-documented for a wide variety of configurations\(^3\)\(^4\); therefore we omitted it as a factor from our study. For reasons of experimental economy we also used only UV photons as input.

When analyzing the experimental design one assumes that the response of interest (or its logarithm, square, etc.) can be well-described by a polynomial with a finite number of terms. In our analysis we include only linear terms; higher order and interaction terms are confounded. While such terms may be significant and distinguishable from noise, confounding prevents their being quantified individually without further trials. For example, lack of fit between the empirical model and the data could be due to a quadratic term in bias angle, or to the interaction between L/D ratio and pore size, or to some combination of the two; it is impossible to say which. Fortunately, statistical design also provides the means to evaluate how well the empirical model performs. In this study we focus on determining the most important effects. Future studies will determine the response polynomial in more detail.

The experimental apparatus comprises a UV deuterium lamp, a resolution mask, an MCP assembly, P20 phosphor screen, and a Princeton Instruments 1024 element PDA with 25 lira pitch pixels (\(<0.001\)^\text{\textasciitilde}). The resolution mask consisted of 0.001" slits separated by gaps ranging from 0.001" to 0.010". The MCP assembly was operated in a turbo-pumped vacuum chamber at a pressure of \(2 \times 10^{-6}\) torr or better.

For the purposes of our study we defined MTF as:

\[
\frac{\text{Peak height} - \text{Valley height}}{\text{Peak height} + \text{Valley height}},
\]

at 13 mm\(^1\) spatial frequency. At this frequency we found the MTF to cover a reasonable range of values, from zero to 0.39.

Figure 1 shows the relative
effect on resolution of the factors we studied. The empirical model described the data reasonably well, having an $R^2$ coefficient of 0.47. Analysis of the residuals showed that the model needs more terms, as we would expect. We believe we understand the role of the terms which we did include. Anode gap is inversely proportional to electric field strength, and consequently controls the time in which the charge cloud spreads. Bias angle also influences charge cloud spreading. L/D ratio probably influences resolution by affecting the energy distribution of the output electron cloud. We plan a theoretical study to investigate this hypothesis. Finally, we find the effect of image enhancement and of pore size to be negligible in this experiment. Note however that the best-performing MCP configuration had a resolution equal to that of the PDA (25 μm); a readout system with better resolution may well have exposed an effect from these factors.

Gain effects are shown in Figure 2. Again, for a screening experiment the empirical model provides a reasonable fit, with an $R^2$ coefficient of 0.49. The influence of L/D ratio and pore size is well-understood: longer pores of smaller diameter provide more multiplying strikes for secondary electrons. Image enhancement also improves the multiplication process, leading to higher gain. Finally, bias angle does not play a large role, but the increase in gain is expected: smaller bias angles provide higher secondary yield on the first strike. The measured increase agrees well with theoretical predictions.

CONCLUSIONS:

For a given MCP to anode spacing one can improve both resolution and gain with a larger L/D ratio and by using the image enhancement process.

The choice of bias angle presents a tradeoff between higher gain and better resolution. For most purposes, however, one can pick a larger angle for better resolution, and then make up for gain losses using a larger L/D ratio.

Pore size may present a similar choice in systems which have a better resolution, but no effect was apparent in our experiment.

Finally, we note that a fully optimized MCP configuration easily resolves features at the limit of the PDA, and thus is not the limiting component of the system.

REFERENCES

A novel tandem quadrupole acceleration-deceleration mass spectrometer

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A tandem quadrupole acceleration-deceleration (QADQ) mass spectrometer for neutralization-reionization studies is described (Figure 1). The MS-1 stage of the instrument consists of an Extrel combined EI/CI ion source, and an EXM-2000 quadrupole filter (Q1) of 2000 a.m.u. mass range. MS-1 is differentially pumped by a 1200 L/s diffusion pump. Mass selected ions are accelerated to 5-10 keV and collimated by a six-element lens (Lens 1) to form a beam of a 4 mm cross section and a 1.5° convergence angle. Lens 1 is furnished with a quadrupole electrostatic lens floated at the accelerating voltage and adjustable to ±120 V to move the ion beam at about 4° space angle. The beam is neutralized by collisions in the first collision chamber (Cell 1), floated at the nominal accelerating voltage and differentially pumped by a 1200 L/s diffusion pump, and allowed to drift through a floated screen-metal conduit to the second collision chamber (Cell 2) positioned 30 cm from Cell 1 and kept at a low potential of opposite polarity to defocus the remaining ions. Cell 2 is differentially pumped by a 60 L/s turbomolecular pump. The neutral products are finally reionized by collisions in the third collision chamber (Cell 3) positioned 61 cm from Cell 1, and floated at a variable high voltage. Cell 3 is differentially pumped by a 1200 L/s diffusion pump. The ions formed are decelerated by a special lens (Lens 2) and collimated to a convergent beam by an einzel lens. The decelerating potential is link-scanned with the final quadrupole mass analyzer. After deceleration the high kinetic energy ion fraction is filtered out by a chicane lens (Lens 3) of a -5 eV energy bandwidth. The optical stop of Lens 3 is mounted on a linear motion feedthrough to allow fine tuning and beam profile measurements. Lens 3 is also furnished with four pairs of deflection plates to aim the beam at the aperture of the MS-2 injection lens. The survivor ion and its reionized fragments are mass analyzed by a second EXM-2000 quadrupole mass analyzer (Q2) as MS-2, and detected by an off-axis electron multiplier furnished with a conversion dynode. The MS-2 part is differentially pumped by a 60 L/s turbomolecular pump.

Figure 1. Ion optics scheme and electrostatic potential diagram of the QADQ tandem mass spectrometer.

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Normal mass spectra are obtained by setting MS-1 in the RF-only mode and scanning MS-2, while keeping the collision chambers and the ion conduit at the accelerating potential. In the neutralization-reionization mode Cell 2 electrically isolates MS-1 and MS-2 and reflects back ions escaping from Cell 1. In the reionization step, the survivor and its reionized fragments are formed from keV neutrals at kV potentials and then decelerated to a low kinetic energy (40-60 eV). Consequently, Lens 3 and the detector can be operated at the ground potential which greatly simplifies both instrument design and data acquisition. Q1 and Q2 are operated at d.c. offsets 2-5 V below the ion kinetic energy in order to maintain high mass resolution.

In preliminary experiments the instrument showed >10% total ion transmission at 4-7 keV acceleration voltage. Unit mass resolution is readily achievable at both MS-1 and MS-2 without severe transmission losses. Relative ion abundances of \(^{124}\text{Xe}\) through \(^{134}\text{Xe}\) were measured by scanning MS-2 at unit mass resolution (<1% valley, Figure 2) and showed good agreement with the tabulated values. The beam cross section, measured at the optical stop of the chicane lens, matches that predicted by ion-trajectory simulations.

![Xe, MS-2 scan, 2 x 10^4 Torr](image1)

![Xe, MS-2 scan, collisional scattering 50% transmittance air, 5 x 10^4 Torr](image2)

Figure 2. MS-2 scans of Xe isotopes.
DESIGN OF AN ULTRA HIGH RESOLUTION ENERGY RESOLVING TANDEM MASS SPECTROMETER

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We have previously proposed [1] ion optical arrangements, comprising a pair of symmetrically positioned electrostatic analysers for achieving very high energy resolving properties in tandem mass spectrometers. An instrument of this design has been constructed and is used to directly measure the electronic and vibrational structure of molecular ions by the technique of Translational Energy Spectroscopy (TES) [2]. In these particular applications, the ion spectrometers are operating with ion beams of kilo electron volt energies (typically 2 – 8 keV) and thus to record electronic and vibrational band structure, very high energy resolving power is required (>20 000). To improve existing instruments to have performance similar to photoelectron spectroscopy, (but for fast ion-molecule collisions), a new TES spectrometer of higher energy dispersion has been designed and is currently under construction.

The basic geometry and associated lenses for this equipment are shown in Figure 1. The principle features of the instrument are the electrostatic analysers which are 0.960 m in radius (an increase of 2.5 on the current instrument) which leads to a similar increase in energy dispersion. These particular sectors were chosen as they were available from a commercial mass spectrometer design. The geometry has been run through the TRIO programme [3] to check that the image aberrations are at an acceptable level (see Table 1). From our experience with our smaller instrument (R = 0.381 m electrostatic analysers) less auxiliary lenses are preferable in the instrument. Einzel lenses are necessary to provide first order focus (Y lenses, Fig. 1) which is a critical parameter to minimise, as the ESA’s are fixed (ie they cannot be moved tangentially to the ion beam, as a magnetic sector can, to achieve first order focus). Hexapoles (H, Fig. 1) are also necessary to carry out minor second order correction and, more importantly, to rotate the image for precise alignment with the collector slit which cannot be mechanically guaranteed. We also plan to include z-focusing Einzel lenses to increase the overall sensitivity as the symmetrical ESA arrangement has no focussing action in the z direction. TRIO indicates a B value of 4.69 for this geometry and therefore use of z focusing would help restore any loss of sensitivity. As this geometry has very low image aberrations for aberrations connected with the height of the ion beam (refer to Table 1 ie YY, YB, BB ....) it is planned to use long slit length (<20 mm to further increase sensitivity. The overall increase in transmission at a fixed energy resolution (compared to the current instrument) is expected to be a factor of ten. The ultimate energy resolution (currently 0.015 eV for a 3000 eV beam, E/ΔE ≤ 200 000) will probably not improve by more than a factor of 2.5 but the increased sensitivity afforded will make measurement feasible at these energy resolutions of 10 meV, allowing photoelectron type spectra of fast ions to be recorded directly.

Data from the current instrument has shown that band structure of ions can be obtained and by varying the target gas singlet, triplet (doublet, quartet) states can be selectively measured [4]. Areas we envisage to investigate are different collision cell designs, in particular, one reported at this meeting where the energy given to the collision gas can be monitored in coincidence with the energy loss of the projectile ion. This method will greatly enhance the resolution of spectra by immediate deconvolution of the energy distribution taken up by the target. Another area in which we have made some preliminary investigations is toward the electronic and vibrational bands of neutral compounds. Figures 2 and 3 show TES spectra for H⁺ on acetone (Figure 2) and H⁺ on propionaldehyde. TES spectra are highly reproducible and the differences between these isomers is easily seen. For propionaldehyde we are picking up a vibrational band at ~-10 eV. By changing the fast ion to another species such as He⁺, N⁺, O⁺ etc. these TES spectra vary significantly allowing a given compound to be mapped. We are currently looking at structural differences down to chiral species and will report these soon.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

**SCHEMATIC DIAGRAM OF 1 METRE TES SPECTROMETER**

**Figure 1**

![Schematic Diagram of 1 Metre TES Spectrometer](image)

**Table 1**

<table>
<thead>
<tr>
<th>Magnet Source</th>
<th>Ion Trap Calculation by</th>
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<th>Beyond Order - Momenta &amp; Energy Separation</th>
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<td>GAP = 0.0500</td>
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**References**

A quadrupole mass spectrometer can be constructed in which only time variant fields are used to produce mass specific detection. This is accomplished by using an r.f. only quadrupole field to which an auxiliary transverse dipole field is added. This auxiliary field is tuned to the natural oscillatory motion of ions near the high q stability limit. Ions in resonance with this stimulus gain transverse velocity and are ejected. The frequency or amplitude of this resonant excitation can be modulated such that the mass specific instability thus produced results in an a.c signal at the detector that is proportional to the ion flux. Phase sensitive detection can then be used to extract mass-intensity information characteristic of a mass spectrum.

There have been many prior implementations of r.f. only quadrupole systems. Many of the drawbacks encountered in prior r.f. only quadrupole systems can be avoided if an alternative method is used to provide the high q cutoff. Ions near the high q stability limit can be selectively excited by application of an auxiliary dipole or quadrupole field. Ions in near-resonance with the excitation field will gain transverse kinetic energy and be rejected if their radial displacement exceeds $r_0$ in less than the time it takes them to traverse the length of the quadrupole structure.

At low values of $\beta$, the ion trajectories may be simply described as a primarily sinusoidal motion. The change in $\beta$ with respect to $q$ is approximately 0.7 up to $\beta = 0.4$ where the slope in this relationship begins to increase. It continues to increase asymptotically until, at the stability limit, where $\beta$ equals one and $q$ equals 0.908, the slope is infinity. Ions near $\beta = 1$ have a motion composed primarily of a pair of frequencies equally spaced on either side of one half of the main r.f. frequency. The addition of an auxiliary field in resonance with either of these frequencies causes the maximum radial displacement of the ion trajectory to increase.

When such a resonance induced cutoff is utilized during a r.f. only mass analysis scan, the observed detected ion current stair steps have steeper transitions than would be otherwise observed. Since the influence of the auxiliary field on ion motion, and therefore ion transmission, is strongly dependent upon differences in the resonant frequency of ions and the frequency of the auxiliary field, small differences in ion masses correspond to large differences in ion transmission. This technique exploits the large value of the change in $\beta$ with respect to $q$ at high $q$ to achieve relatively high resolution with good ion transmission.

Any previously described r.f. only operating mode would benefit by use of a resonance enhanced high $q$ ion transmission cutoff. A further improvement is realized by modulation of the $\beta$ corresponding to the cutoff by modulation of the frequency of the auxiliary field. The detected ion current carried by ion masses which have effective path stability altered by the changing frequency of the auxiliary field will have an a.c component that has a frequency equal to the modulation frequency of the auxiliary field. Mass resolution is a function of the magnitude of the frequency modulation, as this directly determines the range of ion masses that will produce encoded ion current signals.

A simplified block diagram is shown which uses full wave modulation to excite both frequencies of ion motion in quadrature in both dimensions to prevent frequency beating, an obscure but important characteristic of resonant excitation beyond the scope of this discussion. Mixing one half of the main r.f. frequency with an audio frequency produces the needed waveforms. The amplitude is set and inductively coupled to the quadrupole structure. All that remains is to modulate the position or $\beta$ at which resonance occurs, which is accomplished by frequency modulation of the audio frequency generators. The amplitude of the auxiliary field, the chosen average $\beta$, the number of r.f. cycles and the amplitude of the auxiliary field frequency modulation determines the resolution setting of a given device.

MS/MS is possible by using this method to modulate the parent mass.
intensity in the first quadrupole of a tandem instrument. All daughter ions formed in the collision cell will also be modulated. Operating the final quadrupole with a different modulation frequency permits the selective detection of daughters at the appropriate sideband frequencies.

Resonant excitation at low $\beta$ produces notches in the transmission band which may also be modulated. Multiple notches modulated at different frequencies permit simultaneous multiple ion detection. MS/MS with multiple notches in Q1 and Q3 permits simultaneous multiple reaction monitoring.

The most encouraging result is the resolution of mass 1466 using an r.f. quadrupole operated at 1.002 MHz. Similar resolution is achieved in the r.f./DC mode only at the expense of sensitivity. By comparison, the r.f. only mode has more than 50 times as much intensity in terms of ion current at the detector. This result clearly demonstrates the projected advantages of increased sensitivity and resolution at high mass.
In all time of flight (TOF) mass spectrometers it is necessary to measure the time differences between ion production and ion detection. Two methods are mainly used for time measurements, that depend on the number of ions of the same mass striking the stop detector simultaneously. If the output signal from the detector is large a transient waveform recorder is generally used. Most of the time measurements in laser desorption mass spectrometry are performed with such an electronic equipment. Pulse height signals from detectors signal are measured as a function of time over several tens of microseconds with time bins of 5 ns (or much less) according to the frequency of the system (200 MHz or more). Digital values are stored in the computer memory and a complete TOF spectrum may be obtained for only one laser shot. However, several problems may occur such as time precision, uncorrect time-amplitude digital conversion, etc... although excellent commercial devices are available.

When the number of identical ions is very small (around one), single ion counting is more advisable. This is achieved by using a time digital converter (TDC) and a low threshold constant fraction discriminator. The most recent TDC built at the Institut [1] has a time bin of 0.5 nsec and can accept, for one start signal, 255 stops delivered by one or three different detectors (3 stop input signals). The dead time is 22 nsec. A smaller time bin, more stop signals and a smaller dead time will be possible. The time accuracy of a TOF peak centroid can reach 50-60 ps.

Event by event TOF coincidence measurements can be performed with single ion counting. For example time windows can be defined on a time of flight spectrum and used as time origins to generate (during data acquisition) time of flight spectra of correlated secondary ions produced by desorption processes following the main first desorption process. Delayed emission has been measured by this technique [2]. Multidesorption events associated with the same start signal provide a complex TOF spectrum that is the sum of several TOF spectra due to each event. The "time windows" technique allows to obtain these TOF in real time. A TDC, a microprocessor, a DMI card in a PC and a PC386 are used for these data acquisition [3].

Recently a charge digital converter (CDC) has been coupled to a standard TDC. This new device is very useful when several ions of the same mass hit a detector. As soon as a signal from the detector is seen by the discriminator, the total electric charge is measured for a time roughly equal to the dead time of the TDC. The amount of charge is proportional to the number of ions that has hit the detector and a digital value is added in the TOF histogram. It is necessary to measure a calibration curve of the detection system that depends on the energy and mass of the ion and also on both the discriminator threshold and the detector voltage (Fig. 1). Fig. 2 a-b shows matrix assisted laser desorption TOF spectra of a mixture of insulins measured with the TDC alone and the new electronic device. These spectra were recorded with the multidesorption probes TOF 23 ($^{243}$Cf, Cs or cluster ion gun, N2 laser) built at the Institut and commercially available. A double grid mirror is used [4]. The apparent mass resolution in matrix assisted laser desorption is around 2500 in the mass range 1000-2000 compared to 3500 with $^{244}$Cf. At the laser irradiance threshold and with low detection voltage values an apparent M/AM of 1500 is obtained for insulins. The isotopic mass distribution width is in fact the limitation factor. With TOF 23, the neutral spectrum and ion spectrum are recorded simultaneously. It is observed that metastable decay processes are very important in LDMS.

The secondary ion multiplicity can be, in principle, measured with the new CDC-TDC electronic system. Various tests of this module are being made at our laboratory.
Figure 1: Calibration curve for the charge digital converter. The signal amplitude from the MCP, due to impacts of different single ions at 15 keV has been transformed in number of electrons created at the MCP surface.

Figure 2
a. Normal TDC time of flight spectrum of a CsI sample bombarded by Fission Fragments (FF).

b. CDC + TDC TOF spectrum recorded simultaneously. By comparison to Fig. 2.a, it has been calculated that an average number of 2.2 Cs ions is emitted by FF.

Figure 3
a. Laser desorption TOF spectrum of a mixture of insulin measured with TOF23 and a normal TDC. The laser irradiance was at the minimum value.

b. Same measurement with the CDC + TDC converters. The widths of the peaks are about 40 nsec.

A number of schemes have been described which allow the diversion of solvent or major components between the gas chromatograph and the mass spectrometer but most compromise chromatographic resolution or do not really switch cleanly. Because of these problems, many if not most mass spectrometers today utilize the so-called direct connection in which there effectively is no interface. Large components such as solvents are managed by simply turning off the mass spectrometer electronics. Unfortunately, turning off the electronics does not fully protect the spectrometer from the contaminating effects of large amounts of substances.

We have developed an interface which switches cleanly and completely in fractions of a second between a clean helium stream and GC effluent. This is accomplished without compromising GC resolution or sensitivity. In addition to on/off switching the device is also capable of partial splitting. Moreover, the device allows GC column exchange or other chromatographic maintenance without affecting the integrity of the mass spectrometer's vacuum and allows postcolumn derivatization as described in Anal. Chem. 63, 255 (1991).

These improvements are obtained by utilizing a modified open-split design in which the restricting (or inlet) capillary is arranged so that it can be positioned to sample either GC effluent or clean helium. In Figure 1, we provide a schematic of the interface. The inlet capillary is supported by a compression fitting supported on a bellows. This bellows is driven up and down by a mechanical screw arrangement. Clean helium flows into the interface via the bellows and exits via the vent. The flow from a variety of inlets flows through a manifold constructed of 0.3 mm i.d. glass lined tubing and exits through the vent. When the bellows is compressed the inlet capillary samples flow coming from one of the inlets. When the bellows is decompressed the inlet capillary samples only clean helium. The vertical motion required to accomplish switching is less than 2 mm and the time required to switch is less than 1 second. Intermediate vertical positions of the sampling capillary provide partial sampling of the GC flows.

Figure 1. Fast switching interface.
In our prototype the manifold is interfaced to two independent GC columns and to an auxiliary split-type capillary injector which is used to introduce reagents for postcolumn derivatization. The inlets which are not in use are provided with very low flows of helium to eliminate dead volumes.

The advantages of this system include the fact that switching of the interface has no effect on the pressure seen by the outlet of the GC column and no effect on the pressure seen by the mass spectrometer's ion source. As a result switching has no effect on GC retention times and no effect on ion source conditions. Column exchange on this system requires only that the bellows be decompressed after which the GC columns may be changed at will.

Figures 2. and 3. provides examples of the performance of the system.

![Figure 2. Switching between GC effluent and helium while monitoring a GC bleed peak at m/z 207.](image)

![Figure 3. Moving the inlet capillary in small steps between the vertical position which samples only helium and the vertical position which samples only GC effluent while monitoring a GC bleed peak at m/z 207.](image)
INTRODUCTION

In a cryocooler, gas flows in a closed-loop from a compressor through interconnecting line(s) and the regenerator to an expansion volume and, then, returns back to the compressor. The displacer which embodies the regenerator matrix has a temperature range, across the length of its body, from as low as 10 K up to 300 K. Any contaminants which are condensible in this temperature range will be condensed on the surface of the regenerative materials or in the gap between the displacer and its outer enclosure. Consequently, the build-up of the condensate results in a loss of refrigerator performance. Because of the relatively large gas volume being circulated in the system, the concentration of contaminants which is needed to impair the cryocooler performance is usually low enough that they elude detection by any direct analysis technique. A preconcentration step is required.

This paper describes a specially-modified sample inlet system for preconcentrating contaminants in gases from a pressurized system and, subsequently, allowing their detection by the GC/MS technique. The gas volumes being analyzed can range from a few std. mL to tens of std. liters with a corresponding pressure from ambient up to 50 atm. The evaluation of this inlet design was performed on a reference standard gas cylinder which is comprised of a mix of four halocarbons in a balance of nitrogen. The limit of detection for the halocarbons is found to be a few nanograms for the operating conditions under EI in the scan mode. The linear dynamic range extends to a few micrograms being studied. The introduction of this reference standard as an internal standard for semi-quantifying contaminants in cryocoolers is demonstrated.

EXPERIMENTAL

Figure 1 illustrates the block diagram which depicts a sample selection valve, gas switching valves, a cryofocus, mass flow controllers (MFC), and an on-line moisture analyzer. The sample gas volume can be directed solely to the GC/MS or split, with variable split ratios, between the GC/MS and the moisture analyzer. The moisture analyzer, if used, operates under a fixed flow at 100 seem which enables a direct readout of moisture content, in ppmv. The preconcentrating gas flow rates were normally from 5 to 70 seem of helium whereas the desorb flow through the cryofocus was fixed at 5 seem. Various functions can be performed depending on the selected valve position, such as: manifold purging, contaminant preconcentrating in the cryofocus, reference standard and/or internal standard introduction, and contaminants desorbing for GC injection.

The cryofocus, Tekmar Capillary Interface Model 1000, which embodied a deactivated 530 μ fused silica column was cooled to -150°C or below in order to preconcentrate gaseous contaminants. An HP split/splitless capillary inlet injector was modified to be interfaced with this preconcentration column. In the desorb mode, the condensates were flash evaporated, injected, and then refocused at the head of GC column before final elution to the MS detector. Gases exiting from the cryofocus split: one to the GC column with a fixed flow at 0.8 sccm and the other to the split vent with the remainder of flow which is controlled by a mass flow controller. The GC column is a 25 m long by 0.25 mm ID, DB-1 from J&W Scientific. The instrumentation includes an HP 5890A GC interfaced to an HP 5988 MS.

The reference standard cylinder, from Restek Corporation, is comprised of 2.5 μg/μl of chloromethane, vinyl chloride, bromomethane, and chloroethane in a balance of nitrogen. The gas flow manifolds including the sample vessel are kept at 55°C for volatilizing the contaminants.
RESULTS AND DISCUSSION

Table 1 summarizes the results of variable volume sampling of the reference standard. The calculated quantity in Table 1 was derived by multiplying the gas volumes being introduced by the certified concentration at 2.5 μg/μl of the respective halocarbons. Forced zero linear regression of test data indicates that the area response of quant ion vs the quantity of halocarbons is linear, from a few nanograms up to several micrograms being introduced, as shown in Figure 2. The limits of detection, which are defined to be 3 times the signal to noise ratio, are found to be 3, 3, 3, and 8 ng for chloromethane, vinyl chloride, bromomethane, and chloroethane, respectively.

The utilization of this reference standard as an internal standard for the purpose of semi-quantifying the contaminants in cryocoolers is demonstrated in the chromatograms in Figure 3. Figure 3a exhibits contaminants present in a small cryocooler with a static pressure of 50 atm and a void volume of 200 cc. Figure 3b is an example of results from a large cryocooler with a static pressure of 18 atm and a void volume of 800 cc. Future efforts are to replace the use of halocarbon internal standards with a standard gas generator which is capable of providing a known blend of contaminant mix at varying concentrations.

FIGURE 1. DIAGRAM OF ELEVATED PRESSURE INLET SYSTEM

FIGURE 2

Forced zero linear regression of data for variable sample gas volumes

Sample Gas Halocarbon mix in a balance of nitrogen

- - Chloromethane, r²=0.987
- - Vinyl Chloride, r²=0.992
- - Bromomethane, r²=0.997
- - Chloroethane, r²=0.996

FIGURE 3. Chromatograms of gases from two cryocoolers

(a) a small cooler @ 50 atm

(b) a large cooler @ 18 atm

Table 1. Variable volume sampling of the reference standard

<table>
<thead>
<tr>
<th>Sample gas volume (μl)</th>
<th>Relative response factor of quant ion (μg/ml)</th>
<th>Sample gas Halocarbon mix in a balance of nitrogen</th>
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<td></td>
<td>Q (μg)</td>
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Theoretical and Practical Aspects of
Short-Column Gas Chromatography/Mass Spectrometry
Sample Injection Methods

Stacy-Ann Rossi and Richard A. Yost, University of Florida,
Department of Chemistry, Gainesville, FL 32611

Theoretical and practical aspects of different sample injection methods used in short-column gas chromatography/mass spectrometry (GC/MS) have been investigated. It has been shown previously that for short-columns (< 5m), the experimental chromatographic resolution at high carrier gas velocities is not as good as that predicted by theory (1). The differences between experimental and theoretical values can be attributed to extracolumn variances from the sample injection method. If optimum short-column GC/MS performance is to be obtained, the development of a high-speed injection method is necessary to eliminate this extracolumn band broadening. We have investigated the use of Curie-point thermal desorption as a potential high-speed injection method for short-column GC/MS.

Experimental:
All experiments were performed on a Finnigan MAT TSQ45. Sample injection methods including standard syringe injection and Curie-point thermal desorption were investigated. Chromatographic separations were performed on a 4m, 0.25mm i.d. SE-54 capillary column using helium as the carrier gas. Both pressure regulation and flow-control of the carrier gas flow rate were used via a modified gas chromatograph (1). A homebuilt Curie-point unit was used for the thermal desorption experiments with 610 °C wires (2). The first quadrupole (Q1) of the mass spectrometer was scanned over a 2 amu window at a scan rate of 0.05s/amu to minimize the time constant associated with the detector. The anabolic steroid, methyltestosterone, was used in all studies with methylene chloride as the solvent.

Results and Discussion:
Results for standard syringe and Curie-point thermal desorption injection methods were compared to predicted theoretical data in order to evaluate the contribution of these two injection methods to extracolumn band broadening. It was originally believed that the input bandwidth for Curie-point desorption would be narrower than standard syringe injection methods

\[ \sigma_{\text{desorption}} < \sigma_{\text{vaporization}} \]

where \( \sigma^2 \) is the variance associated with the input bandwidth. As shown in Figures 1a and 1b, it is clear that both methods exhibit some degree of band broadening at high carrier gas velocities; however, the syringe injection method appears to yield narrower peak widths than the desorption method.

For the syringe injection method, the extracolumn band broadening results in an additional 0.4s to the theoretical bandwidth at 50% peak height. For Curie-point desorption, the contribution to extracolumn band broadening is three times that of the syringe method, adding 1.2s to the theoretical bandwidth. For plate height comparisons, an additional term \( (Du^2) \) is required in the Golay equation to account for the extracolumn band broadening. In Figure 1b, this is shown as an extracolumn broadening of \( \sigma = 2.5s \) for the syringe injection method and \( \sigma = 5s \) for Curie-point desorption compared to the theoretical plate height (\( \sigma = 0s \)). At this time, we believe that the input bandwidth for Curie-point desorption is not as much a function of the linear velocity, but is limited by the RF power supply used to heat the Curie-point wire to its final temperature. It should also be pointed out that the peaks obtained in these studies were non-Gaussian. To correct for this, the Foley-Dorsey equation was used so that chromatographic figures of merit could be obtained for both ideal and skewed peaks (3).

Considering the practical aspects of these injection methods, Curie-point desorption has been shown to be advantageous over syringe injection methods since there is no solvent background or interferences from solvent tailing, as seen in Figure 2. This allows Curie-point desorption to be used more effectively with isothermal column temperatures since solvent overlap is not a problem. It is also apparent from these chromatograms that for both methods mixture separation capabilities are equivalent and thermal decomposition is minimal; however, interferences from column or septum bleed is evident in the syringe chromatogram but not in the Curie-point desorption chromatogram.
Comparison of Theoretical and Experimental Peak Widths

Theoretical and Experimental Dependence of Plate Height on Average Velocity

Comparison of Short-Column GC/MS Injection Methods:
Temperature-programmed Column Operation

Syringe Injection

Curie-point Thermal Desorption

Figure 1a

Figure 1b

Figure 2

References:
DESIGN OF AN OPTO-ISOLATED DETECTOR FOR MASS SPECTROMETRY

*John W. Gray, Joan M. Green-Cardin, and John B. Hooper
Galileo Electro-Optics Corp., Sturbridge, Massachusetts 01566

The objective of the present work is to demonstrate the feasibility of a practical, cost effective, channel electron multiplier (CEM)-based, opto-isolated detector for mass spectrometry applications, and to identify and characterize the critical operating parameters of such a detector.

Detection of negative ions can be complicated by the fact that the signal is often obtained at high potential. Two methods of dealing with this problem are: 1) use of a conversion dynode to convert the negative ions to positive ions, and 2) pulse counting, which enables capacitive decoupling of the high voltage from the signal.

An opto-isolated ion detector has the advantage of being able to detect either positive or negative ions in the analog mode while still maintaining a signal at ground potential. In addition, it may be possible to float the CEM at a high potential for improved detection efficiency at high mass without resorting to a separate and costly conversion dynode. Figure 1 below illustrates the basic configuration of the device. There are four major system elements important to proper operation:

1) The channel electron multiplier for detecting and converting positive or negative ions to secondary electrons,

2) A phosphor for converting the high voltage electron signal to a photon signal,

3) A single glass fiber for transmission of the photons with minimal loss, and

4) A photodiode to sense the photon signal and convert it to a ground referenced electrical signal.

The photodiode output is directly compatible with most mass spectrometer signal processing electronics and may thus be directly retrofitted into existing systems without modification of the instrument. Two basic mechanical configurations have been constructed: 1) a flange mounted device in which the signal is transmitted through the vacuum housing via the glass fiber and 2) an integrated CEM/collector very similar in appearance to a standard CEM detector in which the photodiode is placed inside the vacuum.

Results of preliminary characterization of system elements will be presented as well as recommendations for future optimization of an integrated detector. The major effort will focus on optimizing the interface between the various components as well as selecting the most appropriate phosphor and photodiode.
OPTO-ISOLATED CEM/MS DETECTOR

**Positive Ion Beam**

-4 to -8kV

PHOSPHOR

OPTICAL FIBER

PHOTODIODE

GND to -5kV

**Negative Ion Beam**

+5kV to +7kV

PHOSPHOR

OPTICAL FIBER

PHOTODIODE

+7 to +9kV

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President Robert Murphy welcomed the attendees and called the Business Meeting to order at 11:00 a.m. He reported that ASMS is a healthy organization with a growing membership. He announced the topics, dates and organizers for three affiliated meetings: The Fall Workshop on "Elemental Mass Spectrometry", November 14-15, 1991, organized by Willard Harrison and Gary Hieftje; the Sanibel Conference on "Lasers and Mass Spectrometry", January 25-28, 1992, organized by Tomas Baer and Klaus Blemann; the Asilomar Conference on "Trapped Ions: Principles, Instrumentation and Applications", September 27-October 1, 1992, organized by Graham Cooks and Alan Marshall.

Secretary Veronica Bierbaum introduced and congratulated the newly elected members of the ASMS Board of Directors: Sanford Markey, Vice President for Arrangements; Brian Chait, Secretary; Alan Marshall, Member-at-Large. She extended special thanks to the other candidates: Ann Goetz, Sharon Las and Jeffrey Shabanowitz. Bierbaum congratulated the recipients of the Student Travel Stipends to the ASMS Conference: Fred Bouchard, University of Waterloo; Bernd Bruenner, University of California-Davis; David Bunk, Texas A&M University; John Elling, University of Wisconsin; Jeremiah Hogan, University of Texas–Austin; Denise MacMillan, University of Nebraska; Ashley McCormack, University of Virginia; Patrick McKeown, University of Delaware; Yuan Mei, University of Florida; Mark Proefke, University of Illinois; Stacy-Ann Rossi, University of Florida; Lynn Teesch, Emory University; Steven Van Orden, University of Arizona; Josie Visentini, University of Montreal; Michael White, Texas A&M University. Bierbaum was saddened to announce the deaths of four long-term ASMS members: Philip Issenberg, Malcolm Dole, Michael Barber and Thomas Aczel. On behalf of ASMS, she extended sincerest condolences to their families.

Treasurer Jane Gale reported that the annual ASMS budget has increased from about $100,000 to $550,000 in the last ten years. The current net assets of $320,000 provide ASMS with financial security and allow the initiation of special projects, most notably the Journal of the American Society for Mass Spectrometry. Gale itemized the income and expenses for 1990 as well as those projected for 1991. There was a net loss of $30,000 in 1990, due primarily to JASMS and the necessity for buses at the 1990 Conference in Tucson. She noted that, since two-thirds of ASMS members attend the annual conference, it is appropriate that conferences represent the major ASMS expenditure.

Kelsey Cook, chair of the Audit Committee, announced that the Auditor’s report for 1989 was convincingly accurate and no irregularities were noted. The audit of the 1990 books has been completed and will be reported to the membership after review by the Audit Committee.

The Vice President for Programs, Henry Fales, expressed appreciation to the many people who contributed to the success of this year’s program. He acknowledged the chairs of the symposia, the oral sessions and the poster sessions, and thanked the members of the Program Review Committee. The 1991 Conference has 315 oral and 632 poster presentations, with about 1850 attendees.

The Vice President for Arrangements, Robert Lattimer, acknowledged the graduate students who served as program assistants. He summarized the results of the 1990 Arrangements Survey which indicated that meeting facilities, the scientific program and reasonably priced accommodations are the most important factors in evaluating the annual conference. Lattimer announced the locations and dates for the next six ASMS meetings: Washington DC Hilton, May 31-June 5, 1992; Seattle Convention Center, June 13-18, 1993 (the 1993 contract with the Las Vegas Riviera Hotel has been cancelled since the planned expansion will not take place); Chicago Hyatt Regency, May 29-June 3, 1994; Atlanta Marriott Marquis, May 21-26, 1995; Anaheim Disneyland Hotel, May 19-24, 1996; Kansas City Hyatt-Westin Crown Center, June 1-6, 1997.

The Member-at-Large for Education, Robert Cotter, acknowledged the members of the Education Committee and the Sanibel Conference Committee. He described the current ASMS Short Courses (Interpretation of Mass Spectra; Advanced MS Interpretation; GC/MS in Drug Testing; Fundamentals of Protein Analysis; LC/MS) as well as courses under consideration for future meetings (Fundamentals;
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Mass Analyzer Basics; Mass Spectrometry in Environmental Issues; Ion Optics; Quantitative Mass Spectrometry).

The Member-at-Large for Measurements and Standards, Michael Bowers, thanked the members of the Measurements and Standards Committee. He reported that the "Standard Definitions of Terms Relating to Mass Spectrometry" will appear in the July-August 1991 issue of JASMS and should serve as a living document. The Committee sponsored a workshop at this conference on the nomenclature for multiply-charged ions.

Past-President Ronald Hites announced that fifteen travel awards have been made to students and postdoctoral associates for the 12th International Mass Spectrometry Conference to be held in Amsterdam August 26-30, 1991: Duncan Bryant, University of Maryland, Baltimore County; David Bunk, Texas A&M University; Kenneth Caldwell, University of Nebraska; Xueheng Cheng, Harvard University; Susan Graul, University of California at Santa Barbara; Steven Hofstadler, University of Texas; Kathleen Kayganich, National Jewish Center for Respiratory Medicine; Jeffrey Klingler, University of Florida; Tapio Kotiaho, Purdue University; Jeffrey Kowalak, University of Utah; Thomas Shaler, University of California at Riverside; Douglas Sholley, Harvard School of Public Health; Lynn Teesch, Emory University; Evan Williams, Stanford University; Susan Wolf, Northeastern University. ASMS and the National Science Foundation each provided $10,000 towards these awards.

The Member-at-Large for Publications, Richard Caprioli, announced that the unit cost of the 1991 volume to the society is $13, representing excellent value.

Murphy then thanked the retiring members of the Board of Directors, Bierbaum, Caprioli and Lattimer, for their service to ASMS and presented each with a plaque. Murphy gratefully acknowledged the continuing support of Judith Watson, Executive Director of ASMS.

The 1991 ASMS Research Award, sponsored by Fisons/VG Instruments, was presented by Lance Nicolaysen to Hilkka Kenttamaa of Purdue University. George Stafford presented the ASMS Research Award, sponsored by Finnigan MAT Corporation, to Chrys Wesdemiotis of the University of Akron.

Murphy announced that JASMS will expand to eight issues in 1991 and that the library subscription rate will be maintained at $250. He thanked Editor Michael Gross for the high quality and excellent value of JASMS.

Murphy then discussed the possibility of initiating a Fellows Program as an avenue for recognizing individuals for their sustained contributions to mass spectrometry and to ASMS. About 8-10% of the ASMS membership would be honored by this program within ten years if there were 75 inaugural fellows and annual induction of 0.5% of the membership. Murphy acknowledged that the program may have negative aspects and be divisive. He welcomed discussion and suggested an informal poll to determine whether a committee should pursue the idea and recommend guidelines. A mail ballot of the membership would be required to amend the constitution and initiate a Fellows Program. Fred McLafferty cautioned that the mechanism of selection is extremely important and that secrecy and continuity must be preserved. Michael Bowers strongly supported the Fellows Program and encouraged ASMS members to applaud the achievements of one another and emphasize a positive spirit; he noted that the APS Fellows Program functions well and benefits the society. Robert Lattimer expressed the concern that certain groups within ASMS would be overlooked; he added that ASMS is a grass roots society that would not benefit from a two tier membership. James McCluskey encouraged the Board to base a decision on the members' wishes. Murphy re-iterated that this is the Board's intention. A hand vote of the attendees indicated that the membership is overwhelmingly opposed to pursuing the inception of a Fellows Program. Chris Enke encouraged the Board to explore other mechanisms for honoring and celebrating the achievements of ASMS members.

The meeting was adjourned at 11:58 a.m.

Respectfully submitted,

Veronica M. Bierbaum
Secretary
Manufacturers' Forum

Have you ever had a flash of insight about how something should be done, but didn't know who to tell your idea to? Or have you ever wondered about how some of the manufacturers feel about a particular topic near and dear to your heart? This year's workshop, sponsored by the Academic Lab Managers' Interest Group, was intended to give you the opportunity to make that earthshaking suggestion, or ask all the manufacturers how they really feel about it.

Larry Mallis (U. of Iowa) organized this year's workshop by contacting 13 primary and secondary mass spectrometry manufacturers to solicit their participation in a Manufacturers' Forum. (Primary manufacturers are the vendors who actually produce mass spectrometers, while secondary manufacturers are those who provide accessory equipment — e.g., chromatographs, parts and services.) Our intent was to provide the atmosphere for a "round-table discussion" in which manufacturers and users could ask questions and trade ideas about perceived problems, general concerns, and suggestions for future development. The forum was neither intended to be yet another sales pitch by the manufacturers, nor a complaint session from users. Representatives from 9 companies attended the forum: Finnigan, JEOL, VG-FISONs, Hewlett-Packard, KRATOS, Hitachi, Perkin Elmer-Sciex, Waters, and Scientific Instrument Services.

The discussion began with each manufacturers' representative introducing himself and his company, and offering a brief statement. A comment made by Finnigan during this opening phase was a statistic attributed to Sun Systems, who report a cycle time of approximately 9 months from release of a product onto the market until it is superseded — and thus is no longer actively supported. This makes it difficult for mass spectrometer manufacturers and users alike to keep pace with computer innovations.

Karl Wood (Purdue U.) asked about policies on support of elderly instrumentation. How long do manufacturers feel the responsibility to support their old products? Hewlett-Packard said their policy is to support a product for 10 years after declaring it obsolete. Their mechanism for deciding to declare a product obsolete is that customers stop buying it in favor of newer products. Finnigan feels both a legal and a moral obligation. The legal obligation is defined absolutely in some countries — e.g., 10 years in Sweden, 5 years in the U.S. Their moral obligation is a compromise between supporting a machine as long as the customer needs and uses it and what is economically feasible.

Larry Mallis (U. of Iowa) asked the manufacturers' feelings on the turn of events following a merger or takeover of an instrument vendor. This is an important consideration if one is in the market for a new instrument or has recently bought one, and is appropriate in light of the fact that four major manufacturers have recently been purchased by larger companies. Finnigan's takeover has resulted in some price adjustments and improvements in production scheduling for the factory. VG's purchase by FISONs marks acquisition by a parent company which appears to be more interested in the scientific instrumentation business than its predecessor. Perkin Elmer-Sciex suggested that their merger would only strengthen the position of the two component companies. KRATOS suggested examining the character of the company itself to assess long-term security rather than trying to generalize about all mergers.

Jack Simpson (NIMH) asked about manufacturers' plans to integrate more completely the control of liquid chromatographs by the mass spectrometer data system, as has been done with gas chromatographs. All manufacturers said they presently have some control, and are working on more.

Bob Minard (Penn. State U.) raised the issue of manuals and schematics. All of us at one time have been more confused than enlightened by a poorly written manual or incorrect schematic. Hank Fales (NIH) emphatically seconded the manufacturers' giving serious consideration of this problem. KRATOS has adopted a policy that new software releases will not be distributed to customers until the manual is ready to go with it. Hitachi, a company re-entering the mass spectrometry market, stated that their manuals are being written in English by native English-speaking authors, not being translated from Japanese. Perkin Elmer countered with the question of how many actually read the manuals. Over 50% in attendance indicated they did!

Tom Karns (U. of Oklahoma) suggested that the manufacturers should be more efficient about informing customers about hardware/software updates, and what new features/fixes are incorporated in those updates. We customers need to know in a timely manner when updates are available, and need the information to make a decision whether an update will improve capabilities sufficiently to justify expenditure of funds. Tom also suggested that, because all the manufacturers depend on computer capabilities, an
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electronic mail connection would be a good communications channel to open. Finnigan reported the availability of an electronic bulletin board at the Finnigan Institute. KRATOS, in addition to their electronic bulletin board, offers a new telephone FAX tablet machine that allows two-way interactive communications that they are trying out.

Hank Fales (NIH) and Sandy Markey (NIMH) made the strong recommendation to the manufacturers, endorsed by the entire audience, that they dedicate themselves more to listening to users' comments and criticisms. The users' group meetings preceding ASMS have become in recent years more a sales pitch and less a problem-solving session. The manufacturers should not feel threatened so much by this kind of dialog. The buyers of their instruments have staked much of their own reputations on these large dollar amount purchase, and don't want the manufacturers to go away. Indeed, they suggest the manufacturers should put more effort into summarizing engineering updates to existing instruments in order to make them work better. Charlie Judson (U. of Kansas) offered the comment, from the viewpoint of a former CEC representative on the receiving end of users' comments, that this type of dialog is extremely productive for the manufacturers. Larry Mallis (U. of Iowa) offered that he likes both the informal one-on-one conferences and the public "gripe" sessions. Both have an important role to play.

Rick Thompson (Merrell Dow Research) asked the manufacturers how they track information coming in from the field on problems and complaints from their service engineers and customers. Hewlett-Packard tracks warranty repair reports very closely to identify recurring faults. Field engineers' reports are computerized and tracked back into the factory as much as possible. Finnigan tracks the number and kind of parts being drawn for service, and tracks telephone support requests and problems. JEOL writes reports on customer comments from demonstrations and training courses. VG and KRATOS have had a relatively small customer support organization in the past and have used communication among the group members, but both are now implementing formalized problem logging and tracking systems based upon H-P's model.

Interest Group Business:

This year's workshop was attended by approximately 75 people. We hoped that the topic would have a wider appeal than to just those affiliated with the Academic Lab Managers' Interest Group. Indeed, a number of people participated who are not directly affiliated with our Interest Group.

Suggestions were collected for future activities for this interest group, and for future ASMS meetings forwarding to the ASMS board. Suggestions for the group were: to continue developing the instrument list; to conduct surveys again for new and changing information; a workshop on hints and tips for evaluating instrumentation. Larry Mallis has reported some statistics from his work on the instrument list, which are given below. Contact him directly for details. Because of my recent change of affiliation from an academic Institution to a pharmaceutical manufacturer, the issue of my finishing my term of office as group coordinator was discussed. Those present agreed to my finishing my term of office.

The ASMS Board has suggested that we expand our purview to include our colleagues who do a similar job in industry. Speaking on behalf of several of our members (including myself) who have crossed this border in both directions, the problems we deal with -- both scientific administrative -- are very similar. I think expansion of our group definition will prevent the group from becoming insular, and further foster the breakdown of the barrier between the academic and industrial worlds. Forthcoming publications from ASMS will show the name of our group as the Analytical Lab Managers' Interest Group, whose stated purpose is to address "problems and solutions encountered by those responsible for mass spectrometry service facilities in both academics and industry."

Reported by Thomas R. Sharp
Interest Group Coordinator

179 Instruments "owned" by individuals on our mailing list (ca. 35-40% coverage)

28.5% VG instruments
21.6% Finnigan MAT (incl. Varian MAT)
19.6% KRATOS (incl. AE1)
14.0% H-P (MSD and research)
5.0% Vestec
2.8% JEOL
2.2% Nermag
1.7% Biolon

1.7% Home-built
1.1% LKB
1.1% CEC
1.1% Sciex
0.5% Hitachi
0.5% DuPont
0.5% Nicolet

1757
Biomedical Interest Group Report

The Biomedical Interest Group meeting was held on Thursday, May 23. The meeting was focused on Strategies for Natural Products Structure Elucidation with ca. 35 people in attendance. The meeting was opened with a request from Steven Carr (SmithKline Beecham), who has accepted the responsibility for chairing the interest group for an additional two years, for suggestions for symposia topics, workshop topics, plenary lecture topics, and possible speakers for any of the above.

After initial opening comments on a general scheme for natural products structure elucidation by Mark Hemling, Tony Thompson (U. Illinois) discussed the utility of high resolution methods. Molecular formula that are generated can be constrained by other available information such as $^{13}$C-NMR or the number and type of heteroatoms determined by derivatization. Selection of the best elemental composition can also be aided by comparing the observed isotope pattern with those calculated for each of the possible compositions. It was noted by Marshall Siegel (Lederle) that computer programs have been described in the literature (K. Blom et al.) which automatically correlate the elemental compositions with the observed isotope patterns to provide a rank ordered list of most likely compositions. With regard to suitable reference standards, the audience was directed to Vol. 193 of Methods In Enzymology (J. McCloskey, Ed., Academic Press, 1990).

The use of derivatization to aid in characterization of natural products was discussed by Jim McCloskey (U. Utah). The topic is so broad that one should go to special sources (Knapp, Handbook of Anal. Deriv. Reactions, Wiley, 1979; Blau & King, Handbook of Deriv. for Chromatog., Heyden, 1978; Anderegg, MS Reviews 1988, 7, 395). While derivatization is used for many purposes, several are of particular value in elucidating structure. These include modification/enhancement of fragmentation to enhance molecular ion yield or to produce structurally informative ions and the identification of specific structural groups. A good derivatization reaction is high yielding with one predictable product which produces an informative mass spectrum. It is also desirable that the product be stable, that labelled reagents are available, that the reaction be microscalable, and that the reagents be commercially available. Two useful derivatizations are trimethylsilylation and methylation. TMS-derivatives are readily made with BSA (BSTFA may be too reactive/unpredictable, e.g., in the presence of CH$_3$CN) although the derivatives of primary amines are relatively labile, especially in FAB matrices. Methylated compounds tend to be fairly stable and are easily made using the Kerek procedure (Ciucanu & Kerek, Carbohydr. Res. 131, 209-217). Hank Fales made several useful suggestions including the use of special narrow bore vials (Wheaton and possibly Peninsula Labs) to prevent the complete evaporation of small solvent volumes and the use of cardboard backed/thick Teflon septa to prevent the leaching of plasticizers from silicon-backed septa.

H/D exchange to determine the number of active hydrogens in an unknown was also addressed by Jim McCloskey. H/D exchange can be done in EI by adding a drop of D$_2$O to the sample on the probe although back exchange can occur with H$_2$O on the source walls. H/D exchange in FABMS/LSIMS is readily done with labelled matrices which are commercially available or may generated as needed by exchange with D$_2$O. H/D exchange in NH$_3$-DCI using ND$_3$ should be done with care as it is possible to exchange aromatic hydrogens and other relatively acidic protons due to the relative gas phase basicity of ND$_3$. The same is true in thermospray. Up to ten hydrogens may be counted directly and computer programs (Sethi et al. or Verma et al.) are capable of dealing with higher numbers by correcting for the extent of labelling in the glycerol peaks from FAB experiments.

A perspective on the proper role for LCMS in natural products structure elucidation was presented by Brad Ackerman (Marion Merrell Dow). LCMS is most generally useful for characterizing similar compounds in a series; otherwise, compounds normally need to be isolated in order to get other spectroscopic data. LCMS may also be useful when chromatophores are not present for UV detection. For molecules below MW 1000, thermospray is acceptable and allows for flow rates in the 1 mL/min range. LC/continuous flow FABMS works for compounds below MW ca. 5000 but sensitivity is compound and mass dependent. LC/CFFAB is amenable to low flow rates and provides a good balance between molecular ion and fragment information. LC/ESI/MS is also amenable to low flow rates with good sensitivity. It may be a nearly universal ionization technique and provides primarily MW information in the normal operating mode. Several examples (castanospermine, telocoplanin) were presented to demonstrate the utility of LC/MS.
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The ability to generate and observe fragment ions from natural products by CAD-MS/MS was discussed by Mark Proelke (U. Illinois). This can be particularly in FABMS when matrix background peaks are obscuring possible fragment ions. Experience in the UI MS lab suggests that CAD with Ar in a grounded collision cell is most useful for compounds of MW > 1000, while He in a floated cell (4 kV) produces the best results for compounds of MW < 1000. As examples of the utility of MS/MS, Mark showed that a suspected glycosylation of an insect neuropeptide was occurring on a Trp residue with relevant fragment ions being shifted the incremental mass of hexose. In the analysis of novel ectinosidins, the similarity of the MS/MS spectra to previously characterized compounds reduced the time required for analysis of the NMR data. In a similar way, the MS/MS spectrum of an unknown derived from a mummy resin was compared to the MS/MS spectrum of the compound proposed from all of the spectroscopic data, and identity was thus established.

Computer tools which might aid in the analysis of natural products were presented by Marshall Siegel (Lederle). These programs can be used to correlate the mass spectrum of compound with a structure proposed from all of the spectroscopic data. Once this has been done, one can more rapidly assign the mass spectra of related compounds. The program takes as input the atoms or superatoms ("indivisible" substructures) of the proposed structure and breaks bonds in all possible ways to attempt to match masses in the spectrum. The output lists the possibilities with the number of bonds that were broken to produce the ion since the most intuitive answer has the fewest number of bonds cleaved. Programs similar to that described by Marshall are available on the VQ Opus data system and from Trinity Software.

As an example of the way various of these methods can be used for analysis of natural products, Justin Stroh (Pfizer) presented the structure elucidation of a spider venom toxin. He pointed out several general issues including a) the need to resolve apparently conflicting data; b) that assumptions are dangerous but necessary at some point; and c) that lack of purity can lead to problems. After analysis of all of the data at hand, a structure was proposed and synthesized. MS characterization of the synthetic material showed that the proposed structure was wrong. Further analysis of the data and reconsideration of certain assumptions resulted in a second structure being proposed and synthesized; this also proved to be incorrect. Reevaluation of the data and the assumptions made and resolution of the conflicting data allowed for synthesis of a final proposed structure which proved to be correct.

Finally, Bob Boyd (NRC, Halifax) presented the bioassay-directed isolation, purification, and characterization of domoic acid, a marine neurotoxin which is occasionally present in mussels in sufficiently high amounts to cause neurological effects including coma and death. This is an elegant example of the power of various mass spectrometric methods when combined with other techniques to rapidly identify the nature of biologically active compounds.

MEETING NOTES

Suggestions for future activities by the Biomedical Interest Group include:

Plenary Lecturers or Topics: Forensic chemistry; NMR spectroscopy (Ad Bax, NIH or Kurt Wutrich, ETHZ, Zurich); posttranslational modifications of proteins (Finn Wold, Univ. of Texas); funding of biomedical research (Bernadine Healy, NIH); frontiers of immunology (John Smith, Merck or Lee Hood, CalTech); chemical mutagenicity and carcinogenicity (Bruce Ames)

Symposia Topics: Mass spectrometry in environmental toxicology

Workshops: No suggestions

Respectfully submitted,

Mark E. Hemling
Steven A. Carr

1759
A workshop to discuss current priorities and goals in NIH funding of academic research, and their real or perceived effects on the academic community of mass spectrometrists, was held on Tuesday evening, May 21. The workshop format was a panel with audience participation. Panel members were the two organizers, Catherine Costello and Charles Coulter, joined by Dennis Bier, Dept. of Internal Medicine, Washington University School of Medicine, Michael Gross, Dept. of Chemistry, University of Nebraska, and Kenneth Standing, Dept. of Physics, University of Manitoba.

Dr. Costello summarized some of the areas that the workshop was intended to cover, and most of them received at least some attention during the following two hours: New ideas and approaches that are becoming available or are on the horizon; potentials for interdisciplinary research; sources for instrumentation funding (Resources, Shared Instruments, Project Grants); effects of funding levels on staff, training, development, access for biomedical and other researchers to state-of-the-art instrumentation, career development of young investigators; grants management and review; methods for effecting changes inside/outside NIH; other sources of support.

Dr. Coulter opened the workshop discussion with a summary of the report "Technologies for the Future: Opportunities and Needs in Structural Biology and Molecular Medicine" which resulted from a recent BRTP workshop. In many areas (MS, NMR, X-ray, 2D gels), this report concludes that the technology presently leads the applications, and recommends that more funding, in the short-term, should be available for applications in the areas of immunology, gene transformations and specific diseases (AIDS, Alzheimer's) than for instrument development. He emphasized the need for program relevance, i.e. a contextual relationship is important - one is better off addressing the problem of 'cellular recognition' rather than 'carbohydrate chemistry.' At the present time, few Study Sections are giving much priority to funding for new major instrumentation. The only funds being approved are those with very apparent program relevance, that is, likely to provide answers to specific biochemical questions, rather than geared toward development of generally applicable new techniques.

Dr. Bier described the NIH MS Resource at Washington University. Here there is much quantitative analysis done to support clinical studies of investigators from many institutions. It is presently difficult to get fixed costs for these studies (support for the instrumentation and staff) from the NIH Divisions that otherwise support the disease-related investigations. He discussed how Core Research (Technological Research and Development) contributes to all other activities, but its role is not perceived by investigators who are interested in solving their research problems by any available means, not interested in MS per se.

Prof. Gross addressed his comments to the review process - specifically the routing of a large number of MS-based proposals to the Metalllobiochemistry Review Committee, a group whose overall composition is not appropriate for review of MS proposals. He suggested that a Bioanalytical Chemistry study section be formed. (This suggestion was heartily approved by most attendees.) Mike also pointed out that NSF no longer funds Resources. Attitudes within the NSF Biology Division presently seem more progressive and open to new approaches than are those of Chemistry.

Prof. Standing commented that the 2-year funding cycle leaves only a narrow time window for an investigator to demonstrate the success of a project. Longer grants, especially when instrument development is involved, would be more practical for both agency and grantee. The Canadian system tends toward longer-term funding of individual researchers.
The general discussion involved panel members and many in the audience, which numbered 60-160 participants during the course of the workshop, and brought out several themes: (1) Instrumentation funding without concomitant staff support is not cost-effective nor is nursing old instruments beyond their reasonable lifetimes. (2) The mass spectrometry community as a whole - users and manufacturers alike - does little in the way of public relations to advance its own cause, when compared to other groups - we should become more active and more sophisticated in focusing the attention of NIH administration, Congressional members and staffs, and our biochemical collaborators on the requirements for consistent support for major instrument development, purchase, and operation, and on making our contributions visible outside the MS community. (3) More active involvement of the manufacturers' group in ASMS through formation of a committee having its own agenda and open to considerations such as the sponsoring of fellowships, etc., might be helpful to all. (4) Some academic support is available through fee-for-service work for local industry, but one must take care to avoid competition with commercial laboratories and to include a research component if academic-industrial projects are included in NIH funding requests. (5) Interest is high for involvement of mass spectrometrists in a future Workshop similar to the one described for Structural Biology. (Omission of mass spectrometrists from inclusion in the Structural Biology workshop was a bit disheartening, but perhaps future programs will compensate.) (6) In some areas, technology does not presently lead the needs of the pharmaceutical industry. The pharmaceutical industry, in particular, has problems for which existing technology is inadequate. (7) Remedying the situation with regard to the Metallobiochemistry Study Section should receive very high priority, with members, including the ASMS board, encouraged to take an active role in effecting change.

Item (2) struck a responsive chord with many attendees and was the focus of numerous post-workshop discussions. Most seemed to feel strongly that ASMS should be looking into ways of collaborating with other science societies to promote, via lobbying efforts, research funding in the US.
An audience of about 170 attended the workshop of the Desorption Ionization interest group, which focussed this year on the subject of sample manipulation in FAB mass spectrometry. Following a few opening remarks, Richard van Breemen of North Carolina State University discussed some of his work with nitrocellulose films in the FAB determination of peptide mass spectra. Monolayer coverage of the nitrocellulose as a film deposited from an acetone solution onto the FAB platform was optimal. Too much nitrocellulose actually increased the level of chemical noise in the mass spectrum. Variation of the sample platform material from gold to copper to stainless steel did not produce any spectral effect. The nitrocellulose may act to prevent the irreversible adsorption of the sample molecule on the metal surface, and aid in the dissolution of the sample in the FAB solvent matrix which is applied after the sample has been applied to the film. Nitrocellulose also seems to act to reduce the extent of (M+Na)+ ion formation, although the cause of this effect is not yet clear. The signal-to-background levels in the mass spectra of several peptides were shown to increase with the use of the nitrocellulose surface modifier; absolute signal levels did not change appreciably. The nitrocellulose used in this work was obtained from Hercules, and dissolved in an acetone solution at a concentration of about 1ug/ul. Nitrocellulose from other sources may contain modifiers and surfactants. As a result, it may not dissolve completely, or the presence of the wetting agents may corrupt the FAB mass spectrum.

Susan Richardson of the US Environmental Protection Agency in Athens, GA discussed the use of liquid secondary ion mass spectrometry (FAB) in the negative ion mode for the determination of sulfonated azo dyes in wastewater. These dyes were either mono or di-sulfonated. The dyes were purified by liquid chromatography and prepared as standard solutions in a number of solvent matrices, including meta-nitrobenzylalcohol, glycerol, thioglycerol, diethanolamine, hydroxyethyldisulfide (HES), and a mixture of HES and thioglycerol, and the eutectic mixture between dithiothreitol and dithioerythritol. Comparative rankings of the signal intensities for the (M-Na)- ions of the dyes investigated showed that nitrobenzylalcohol was the preferred matrix for these compounds. A molar ratio of 100-200/1 provided the best signal quality, and in most cases, solubility of the dye in the matrix seemed to be the limiting factor. The effects on the mass spectra of changes in the primary ion energy (a cesium ion gun was used as the primary particle beam source) showed that for most of the dyes, a beam energy of about 10-15 keV provided the best signal, and this optimum energy was about the same for both the lower and the higher molecular weight dyes. Discussion from the audience suggested that the more highly sulfonated dyes do work better in the diethanolamine matrix than in the nitrobenzylalcohol, although the sensitivity is lower than might be expected for compounds that exist in the solutions as pre-ionized species.

Ken Busch presented the brief story of FAB flakes, which is the characterization of the product formed on the surface of meta-nitrobenzylalcohol on irradiation by the primary particle beam. This project was the research project of F. A. Byrdy, who first characterized nitrobenzylalcohol as obtained from the manufacturers (it includes trace levels of aldehydes and several other related compounds, as well as BHT anti-oxidant), and then used FAB in a synthetic mode to prepare and collect several hundred mg of the product. No FAB spectrum could be obtained, nor could a solvent be found in which NMR spectra could be measured. It was concluded that the product is the result of a condensation polymerization reaction (loss of water) between molecules of the nitrobenzylalcohol. It was noted that the use of NBA in a continuous flow FAB probe can lead to plugging of the line, presumably due to formation of this polymer at the tip of the entrance capillary.

Respectfully submitted,

Kenneth L. Busch
The Environmental Applications Interest Group held its annual workshop on Wednesday, May 22 at 1:00 pm. The topic for this year's workshop was "Methods for Environmental Field Analysis" which dealt with the application of transportable mass spectrometers for on-site environmental monitoring. Various aspects of field analysis were addressed during short presentations by five different invited speakers. Overall, the workshop was well attended with approximately 120 participants. The workshop speakers and the topics of their presentations are listed below.

Stephen Billets of U.S.E.P.A. Las Vegas, NV.

Presented a summary of the EPA Superfund Innovative Technology (SITE) Program and talked about the need for new technologies which could be used for rapid field screening and site characterization. Discussed the potential role of mass spectrometry as a tool for on-site environmental measurements and encouraged ASMS members to play an active role in this endeavor.

Phil Hemberger of Los Alamos National Laboratory, Los Alamos, NM.

Discussed the development of a transportable GC/Ion Trap Detector and its application to the on-site measurement of volatile organic compounds in water. Emphasized the successful use of commercially available components as opposed to the need to custom design and build instruments for field use.

John Wronka of Bruker Instruments, Billerica, MA.

Talked about actual results from the evaluation of a mobile quadrupole mass spectrometer at an EPA Superfund site. Results which were reported demonstrated that a mobile mass spectrometer could indeed perform rapid screening and quantification of a variety of different target analytes and compare favorably with established laboratory procedures.

Brian Eckonrode of Viking Instruments, Reston, VA.

Provided a summary of the capabilities of a new portable GC/MS (quadrupole) instrument for environmental monitoring applications. Discussed the performance of a thermal desorption sampling interface for semivolatile compounds.

Greg Hurst of Oak Ridge National Laboratory, Oak Ridge, TN.

Reported on the use of a direct sampling ion trap mass spectrometer for the rapid screening of volatile organic compounds in water and soil. Presented results showing that screening and quantification of target analytes could be accomplished at low part-per-billion levels at less than 3 minutes per sample by performing direct purge into the mass spectrometer.

Submitted by:

Marcus B. Wise
Oak Ridge National Laboratory
The 39th Annual Conference saw a lively interest in the topic of quadrupole ion traps, beginning with the plenary lecture of Professor Paul, Bonn, and continuing with a number of presentations which covered improvements in ion isolation, mass resolution, combinations with chromatography and simulations of ion motion. This made it particularly appropriate that, late in the week, there should be a workshop devoted to the topic of fundamental aspects of ion traps. Interest in the topic was reflected in the fact that approximately 250 people attended.

The workshop started with a discussion of current capabilities of the instrument in chemical analysis, Rick Yost reviewing scan modes and performance capabilities in a variety of applications. Some figures of merit were then discussed, with considerable audience participation. Included were questions of dynamic range, injection efficiency, CID efficiency, ion temperatures and alternative activation methods.

Perhaps the topic of most intense interest was that of ion motion in the trap. Hans-Peter Reiser described results of a simulation program which displayed ion motion and Randy Julian discussed a large scale simulation which includes the effects of space charge and collision dynamics. Michel Weber-Graubau, John Todd and several others also contributed to this discussion.

The organizers appreciate the fine response, especially the audience participation in this workshop.

Graham Cooks and Rick Yost
In the first 5 months of 1991, over 130 papers dealing with the chemical and physical properties of buckminsterfullerene and its congeners were submitted for publication, corresponding to an incredible rate of nearly one new report per day. The flurry of research activity dedicated to this new form of carbon comes on the heels of the discovery by Krätschmer and Huffman that bulk quantities of C_{60} and other fullerenes could be easily extracted from synthetic soot. The physical characterization of these new materials is a vital task that has rallied together researchers from otherwise disparate corners of chemistry. Mass spectrometry continues to play a key role in these efforts, and it is therefore entirely appropriate that the ASMS provide a forum in the form of this workshop for presenting new results related to the mass spectrometry and gas phase chemistry of fullerenes.

Steve McElvany of the Naval Research Lab inaugurated the workshop with a brief chronology of progress in fullerene research since Smalley first proposed the "Bucky-ball" structure for C_{60} back in 1985. A listing of reviews and current achievements in this field is available through INTERNET at the e-mail address below:

BUCKY@SOL1.LRSM.UPENN.EDU

A. G. Marshall, J. V. Coe and co-workers at Ohio State University and Bob Hettich and his colleagues at Oak Ridge National Lab independently reported the observation and identification of the doubly-charged fullerene anions C_{60}^{2-} and C_{70}^{2-}. Both groups utilized laser desorption of fullerene extracts from synthetic soot in conjunction with FTICR. The identity of these ions was verified through a combination of high resolution mass measurements, double resonance ejection experiments, and by comparing frequency shifts for singly- and doubly-charged ions as a function of ICR trapping voltage. The origin of the doubly-charged ions was proposed to be thermionic emission from the laser-heated graphite surface rather than multiple electron attachment in the gas phase.

Lee Sunderlin and this author in collaboration with Bart Kahr and Dor Ben-Amotz of Purdue University described the gas phase reactivity of C_{60} and C_{70} under flowing afterglow conditions. These ions react slowly, if at all, with O_{2}, the strong Lewis acid BF_{3}, the strong Bronsted acid CF_{3}COOH and NO. However, both fulleride anions do react efficiently with NO to form the monoadducts.

Mark Ross summarized the past and on-going studies of carbon cluster chemistry at NRL using FTICR and other methods. Recent research on fullerenes has yielded measurements of ionization potentials, proton affinities and information about unimolecular and bimolecular reactions. In particular, CID of multiply-charged fullerenes produces lower charge-state fragments as well as charge-stripping and electron capture. Surface collisions of fullerene ions are found to be surprisingly ineffective in producing fragmentation.
R. Graham Cooks, Bart Kahr and Dor Ben-Amotz of Purdue described the higher mass fullerenes that can be observed from soot extracts under El and CI conditions. Satellite peaks at 14, 15, 16, 17 mass units higher are observed for C_{60}, C_{70} and other clusters; the M + 16 signals are attributed to monoxygenated cluster ions. It was proposed that these carbon cluster derivatives are created during the arc-welding process and/or during solvent extraction of the soot. Irradiation of the benzene extract from the synthetic soot yielded sequential addition of methylene groups to the oxygenated clusters, while irradiation of ether extracts produced multiple oxygen atom additions.

Alex Harrison and colleagues of the University of Toronto reported that collision-induced dissociation of C_{60}^+, C_{70}^+, C_{72}^+ and C_{74}^+ occurred by loss of an even number of carbon atoms, in agreement with photodissociation studies of C_{60}^+ and C_{70}^+. By contrast, upon collisional activation C_{60}^+ and C_{70}^+ fragment by loss of 1, 2 and 4 carbon atoms. Collisional charge inversion of C_{60}^+ and C_{70}^+ leads to positive ions which fragment by loss of an even number of carbon atoms.

In a collaborative effort, Helmut Schwarz, Thomas Wieske (Berlin), Wolfgang Krätschmer (Max Planck), and Diethard Bohme (York University) carried out high-energy (8 keV) CID experiments with C_{60}^+ and C_{70}^+ in a four sector instrument. With most common target gases (H_2, D_2, Ar, Ne, etc.) the familiar C_{60}-loss fragments were observed. However, with He targets new signals appear 4 mass units higher than each of the C_{60}-loss products, and with "He [M - C_{60} + 3] signals are observed. Analogous results were obtained with C_{70}^+ . These results clearly indicate that a helium atom has been incorporated into the carbon clusters in the course of the collisions. These remarkable observations constitute the first evidence for formation of endohedral cluster compounds from a bimolecular reaction.

Kenny Caldwell and Michael Gross (University of Nebraska) in collaboration with Sam Hsu (Exxon) reported on the measurement of the second ionization energy of C_{60} obtained by charge stripping. A value of 12.5 ± 0.5 eV was obtained. The curious doublet for the loss of C_7 (MIKES scan) was also noted and explained as formally being loss of C_7 and (C_7 + He) from the parent (or precursor) ion, consistent with the results from Schwarz’s laboratory in Berlin.

Mike Bowers (UC Santa Barbara) described a new ion chromatography method that can separate geometric isomers of ions, and its application to positively charged carbon clusters formed by laser ablation. The method relies on the measureable differences in mobility of ions with different shapes. Seven different families of carbon cluster cations have been observed. Small clusters (n > 10) tend to be linear; intermediate size clusters (n = 10 to 40) are primarily two-dimensional ring structures; larger clusters tend to be fullerenes (n > 40 for n even), although odd clusters retain substantial fractions of planar structures above C_{60}^+ .

Meeting Notes: Before beginning the workshop, suggestions for future plenaries and symposium topics that fall within the "Fundamentals Interest Group" purview were solicited from the gathering (75 - 100 attendees). Topics of interest for next year include metal ions, ion spectroscopy and interstellar chemistry. Additional suggestions may be sent to me at Purdue.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

Workshop for History of Mass Spectrometry Interest Group

There were two announcements of general interest made before the more formal part of the program. First, Mike Grayson announced the completion of the oral history he has made from conversations with A.O. Nier. The document looks marvelous. Second, George Waller announced that his donation to the Smithsonian of the last remaining prototype of the LKB 9000 mass spectrometer, out of 3 that were originally made, has been accepted by that institution.

The interest group organized a workshop on "Oral History: What It Is and How to Take One" on Monday evening of the meeting. The workshop was in the form of two sequential presentations, both made by Dr. James Bohning of the National Center for History of Chemistry, of which the ASMS is an affiliated member. Dr. Bohning was trained in chemistry, but became interested in the history of chemistry, developed techniques in taking oral history and finally, in 1990, retired from a 30 year teaching career at Wilkes College to join the NFHC Staff as Assistant Director for the History of Chemistry. His first presentation was a definition of Oral History as well as excerpts from interviews with prominent chemists which he or his colleagues at NFHC have conducted. Oral history, as defined by Jim and in brochures from NFHC, is a tape recorded structured conversation that attempts to construct an "orchestrated autobiography" of the narrator. The results are intended to be part of the historical record and available for future use by researchers. Interviews are designed to obtain insights into what lies behind the public face of science that is preserved for posterity in publications, etc. Family background, factors that influenced career decisions, personal recollections that are tied to publication of experimental and theoretical work, etc. are all appropriate topics for the interview. Each interview is designed to integrate the personal aspects with public aspect of a career, and to place both within the context of broad developments of science.

The second presentation that Dr. Bohning made was directed toward introducing people to the techniques of taking an oral history. The goal of this presentation was to begin developing a small pool of ASMS members who will be willing to do oral history interviews, as Mike Grayson has completed with Al Nier and has begun with Sy Meyerson. Jim made this presentation by walking the audience through a set of notes he has prepared. The notes outline the interview process in terms of preparation, equipment necessary, areas to cover in the interview,
guidelines for intelligent listening, transcription and editing of the taped interview, obtaining final documents and releases, etc. This remarkable instructional period was followed by questions.

As Jim Bohning said during his presentation, the major developments in mass spectrometry are relatively recent, and thus The History of Mass Spectrometry Interest Group, and the Society as a whole, have a unique opportunity to capture history from its living participants. He feels that if the ASMS can develop a list of of potential interviewees that is mutually acceptable to both the Society and to the NFHC, then the NFHC and the staff of the Beckman Center for History of Chemistry will do the transcriptions of the interviews, with the interviewer doing the subsequent editing. (As Mike Grayson will gladly verify, the transcription is a major component of any oral history, and the NFHC's offer to perform this task could represent the difference between an effective oral history project in the ASMS or having no project at all.) Completed, edited interviews would be integrated into the Center's catalog of oral histories. In addition to several people stating interest in participating in such projects, and proposing names of potential interviewees, it was the sense of the workshop that the ASMS should solicit names of potential interviewees from the membership at large with the goal of developing the list proposed by Dr. Bohning. At the present time, it will be proposed to the Board that this solicitation be made in the fall issue of the Spectator. Copies of the outline notes for conducting interviews may be obtained by request from:

Al Yergey  
NIH  
Building 10, Room 6C101  
Bethesda, MD  20892  
301/496-5531

Signed:  
Alfred L. Yergey, Chairman of Interest Group on History of Mass Spectrometry.
A brief business meeting and the workshop were held on Tuesday, May 21 at 8:00 pm. About twenty people attended. Attendance was limited because many of the group’s members attended the Alfred O. Nier Symposium two weeks earlier and did not come to the ASMS meeting.

The workshop addressed the precise measurement of noble gas isotopic ratios. Philippe Chastagner, Westinghouse Savannah River Co., reported that there is increasing emphasis on the development of methods of measuring the concentrations of the stable fission product isotopes of Kr and Xe that are precise and accurate within ±20 ppm. These measurements are needed for: monitoring atmospheric dose levels; nuclear emergency response; treaty verification; and meteorological transport studies. Current analytical limitations include isobaric interferences, "memory" problems and lack of agreement on the global isotopic composition of natural Kr and Xe.

John Wacker, Pacific Northwest Laboratory reported on experiments in which degassed quartz was exposed to noble gas mixtures (0.02% Kr & Xe in 32% Ar, balance Ne) for 24 hours at 25°C and atmospheric pressure following which the gas was pumped out. After one hour of pumping, the quartz retained 0.3% of the gas-phase Xe and ~10 ppm of each of the other gases. Baking at 1000°C was required to reduce the trapped gases to background levels. Following these tests, he redesigned his equipment and used borosilicate glass, Inconel and stainless steel, materials that had lower gas retention than quartz. And, he followed rigorous cleaning and baking procedures to reduce the blank level to acceptable levels.

Richard Rankin, Idaho National Engineering Laboratory, reported that the Westinghouse Idaho Nuclear Company (WINCO) Gas Analysis Lab performs an average of 130 high precision noble gas analyses per month with a hybrid Finnigan MAT 271/251 gas mass spectrometer. Sample sizes range from 0.07 to 4 cm³@STP and are contained in breakseal tubes. The instrument is equipped with a dual viscous flow, ratioing inlet system and 3 ion collectors. In this system, the samples are compared with a laboratory reference sample - a large supply of which is available. Internal precision has been as good as 10 ppm. Typical sample analysis precision ranges from 20 to 50 ppm.

Submitted by Philippe Chastagner, workshop organizer.
Nomenclature for Mass-to-Charge Ratio

A Workshop Sponsored by the Measurements and Standards Committee

The Measurements and Standards Committee sponsored a workshop on nomenclature for mass-to-charge ratio. After a welcome by Michael Bowers, Alan Rockwood gave a short presentation. This was followed by an open discussion. At the end of the workshop, participants returned an informal survey which has been forwarded to the Measurements and Standards Committee for further consideration.

It was proposed that mass spectrometrists adopt a unit for mass-to-charge ratio to be called a thomson (in honor of J. J. Thomson) or some other suitable name. The $^{12}$C$^+$ ion would have a mass-to-charge ratio of $11.9994514198$ thomsons and the $^{12}$C$^-$ ion would have a mass-to-charge ratio of $12.0005485802$ thomsons. Thus, the thomson would have a value of $1.0364272 \times 10^{-8}$ kilograms/coulomb with the polarity of the ion included in the scale. A proposed abbreviation for the thomson was also discussed, and it was pointed out that the originally suggested abbreviation, Th, conflicts with the abbreviation of thorium so something like Tn might be a better choice.

Supporters of the nomenclature proposal generally felt that present nomenclature has encouraged (or at least failed to discourage) imprecise usage, particularly the use of terms related to mass when mass-to-charge ratio is meant. In part this may be attributed to the lack of a convenient name for a unit of mass-to-charge ratio. Imprecise usage may lead to faulty communication or worse, particularly when one is dealing with multiply charged ions. In the past this imprecision has not been a great problem because multiply charged ions were relatively uncommon, but with the advent of techniques capable of producing tens or even hundreds of charges on an individual ion the distinction between mass and mass-to-charge ratio must be more strictly maintained. For example, according to one anecdote given at the workshop, the confusion between charge and charge-to-mass had led to false conclusions about the upper molecular weight limit of a particular analyzer when used with electrospray ion sources. It was argued that defining and naming the thomson as an explicit unit of mass-to-charge ratio would help enforce a strict distinction between mass and mass to charge ratio and lead to clearer and more concise communication. A second reason for supporting the proposal is that given the central role of mass-to-charge ratio in the field of mass spectrometry as the quantity actually measured in mass spectrometers, it makes sense that the unit for this quantity be given a convenient name.

Opponents of the proposal countered that $m/z$ is already a very clear, well defined and convenient terminology. It was also pointed out that the "thomson" is not self defining, that like the hertz, the units are not explicitly conveyed by the name. This could confuse some readers, particularly the uninitiated. (Whether $m/z$ is a self defining unit was not discussed, but at least it has become understandable through wide and long usage). It was also pointed out that imprecision is not inherent in present nomenclature and that by a combination of present nomenclature and careful language one can write and speak without ambiguity, although at times some extra wordiness might be required.

Both sides presented good reasons for their respective positions, and this seems to have been reflected in the vote. Forty-seven response forms were returned with 57% in favor of the proposal, 34% opposed, and 9% uncommitted. (Not counted in the balloting were letters of support from two editors, and the moderator (ALR) clearly advocated the proposal but didn't vote.) Regardless of the positions on the proposal itself, there was widespread agreement that imprecise communication is sometimes a problem and that mass spectrometrists should be encouraged to avoid incorrect or ambiguous usage.
A surprising part of the workshop was a widespread disagreement on the correct dimensionality of \( m/z \). Three opinions were expressed. The view of the workshop leader going into the workshop was that \( m/z \) represents mass-to-charge ratio so the proper dimensionality would be mass divided by charge. This would be the correct dimensionality to use in the equations of motion for a charged particle in a mass spectrometer and it would be analogous to the usage in tables of fundamental constants in which (for example) the charge-to-mass ratio of the proton \((e/m_p)\) is given as \(9.578309 \times 10^7\) coulombs per kilogram. This is also closely related to the terminology of "grams per equivalent" and "equivalent weight" from electrochemistry. A second more popular view was that \( m \) is a mass but \( z \) is a pure number (being charge number, not charge), so \( m/z \) would have dimensionality of mass. A third view held that both \( m \) and \( z \) are dimensionless so \( m/z \) is a dimensionless number. The official definition of \( m/z \) is that it is a dimensionless number that is proportional to the charge-to-mass ratio. In this respect it somewhat resembles other dimensionless numbers such as reduced parameters from thermodynamics (e.g., reduced temperatures) and dimensionless groups from engineering (e.g., Reynolds numbers). Calling \( m/z \) the mass-to-charge ratio is a bit of convenient linguistic shorthand that is not strictly correct. (A subtle difference between the thomson and \( m/z \) then would be that the thomson has the dimensionality of mass/charge while \( m/z \) is dimensionless, although in magnitude the two are identical.) This widespread disagreement on the correct meaning of such a widely used symbol as \( m/z \) indicates a possible need (or opportunity) for an effort in education or self education. The nomenclature summary that will soon appear in J. Amer. Soc. Mass Spec. is a significant effort in this direction, and it should be read by all mass spectrometrists.

In one of the lighter moments of the workshop, it was pointed out that a natural form to plot electrospray mass spectra would be intensity versus charge-to-mass ratio (rather than the mass-to-charge ratio) resulting in almost evenly spaced peaks. The unit of charge-to-mass ratio could be called a nosmoht which is thomson spelled backwards. (This is analogous to the unit of inverse resistance, the mho, which is ohm spelled backwards.) However, support for the nosmoht appeared to be minimal.

Submitted by:

Alan L. Rockwood
Battelle, Pacific Northwest Laboratory

Young Mass Spectrometrists Interest Group

The topic for the Sunday evening workshop was "Successful Grant Writing." This topic was covered in the workshop four years ago, but the increasing difficulty in obtaining funding made the subject appropriate to revisit. The workshop was well attended by both young and "more mature" mass spectrometrists. Five speakers gave excellent presentations which are summarized below.

Jon Amster from the University of Georgia gave an overview of the technical aspects of grant writing. He prepared two tables, shown on the next page, that detail the overall budget, review process, typical funding level, and hit rate (percentage of grants funded) for most funding agencies. He described the benefits of grant writing, especially the focus it brings to one's research ideas. Jon suggested applying for smaller, higher hit-rate grants during the first few years to gain experience in grant writing and to generate publications that will help in obtaining larger funding from agencies such as NSF or NIH.

Jennifer Brodbelt-Lustig of the University of Texas at Austin gave tips on things to do: give details on planned experiments, be focused on a specific problem, show a time line for progression of experiments, show preliminary results, and give thorough literature citations. Have a friend who has successfully received funding critically read the grant. Study carefully comments from past grant reviews. Finally, try to maintain a high degree of persistence, resilience, and humility.

Mark Ross of the Naval Research Lab gave the perspective of a program reviewer for the office of Naval Research. He emphasized that the major, well-known funding agencies are only part of the funding sources. He highly recommended a booklet published by NSF, "Grant Opportunities for Chemists," as a valuable resource for identifying sources of funding. This booklet may be obtained by writing to the Director, Chemistry Division, National Science Foundation, Washington, DC 20550. Mark gave an outline of an ideal proposal and emphasized that proposal guidelines should be followed closely. He was adamant about not exceeding page guidelines; excessively long proposals have much lower chances of favorable review. He reiterated the need for focus in the proposal, presentation of key experiments, and (for certain granting agencies) the relevance to the agency's mission.

Robert Cotter from the Johns Hopkins University gave the perspective of both an experienced grant writer and reviewer. One should make sure a grant is submitted to the appropriate agency and program, then check to make sure it is really received for review at the proper program. The directions for proposal preparation should be carefully followed, and each section should be distinct; each section should not contain the same material reworded. It is important to keep colleagues (potential reviewers) informed of your research so they can better judge your proposal. Finally, don't burn bridges -- the person whose work you criticize may be a reviewer.

Brian Musselman of JEOL gave suggestions for budgeting and obtaining quotes for grants that request funds for building, modifying, or purchasing instrumentation. He emphasized the need to make sure that all costs are included (e.g., even nuts and bolts where appropriate). Furthermore, he suggested that it is important to state explicitly when contributions are made by a university or department, e.g., institutional funding, machine/glass shop, etc.

Although it was acknowledged the funding is difficult and often frustrating to obtain, there are many ways, some given here, to increase one's chances of getting a piece of the pie.

Respectfully submitted,

John T. Stults,
Genentech, Inc.
The 39th ASMS Conference on Mass Spectrometry and Allied Topics

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Proposed budget figures for the 1992 fiscal year
Source: C&E News, Feb 18, 1991

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<td>$70,000/yr direct 5 yrs.</td>
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<td>DOE</td>
<td>$100,000/yr 3 yrs.</td>
<td>20%</td>
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<td>DOD - ARO, ONR, AFOSR</td>
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</tr>
</tbody>
</table>

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<td>TOM KARNS</td>
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<td>DANIEL B KASSEL</td>
<td>GLAXO, INC</td>
<td>FIVE MOORE DRIVE</td>
<td>919 941 3512</td>
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<td>Milton I Levenberg</td>
<td>ABBOTT LABS</td>
<td>DEPT 418, AP9</td>
<td>708 937 2080</td>
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<td>David Levine</td>
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<td>W V LIGON</td>
<td>General Electric Company</td>
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### 1991 ASMS Conference Attendees

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<td>Johns Hopkins University</td>
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<td></td>
<td></td>
<td>725 North Wolfe Street</td>
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<td>MARGARET MORELAND</td>
<td>Lockheed ESOC</td>
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<td>Geophysics Laboratory/LID</td>
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<td>CURTIS D MOWRY</td>
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<td></td>
<td>Rochester NY 14623</td>
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<tr>
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<th>Name</th>
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The 39th ASMS Conference on Mass Spectrometry and Allied Topics
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<th>Position</th>
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