

## The Mass-Changing Reaction Between MS and MS

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Tandem mass spectrometry (MS/MS) has achieved a very high level of importance in the field of mass spectrometry. In the analysis of targeted compounds in complex mixtures, the first mass analyzer is used to select ions of the targeted compound's mass and the second mass analyzer provides the targeted compound's mass spectrum or detects an  $m/z$  value in that spectrum. Much more information concerning the structure of pure compounds is provided by obtaining, with the second mass analyzer, the mass spectrum of each of the  $m/z$  values appearing in the mass spectrum from the first mass analyzer. These common conceptualizations and applications of tandem mass spectrometry, and indeed the nomenclature of MS/MS instrumentation itself (BE, QQ, etc.), tends to concentrate on the types of mass analyzers employed and appears to take for granted the essential process occurring between the mass analyzers. It is clear that without some mass changing (or, more rigorously, mass-to-charge-changing) reaction after the first mass analyzer, the MS in the second mass analyzer stands for "more of the same". This presentation reflects on the historical significance of the recognition of low-energy collision-induced dissociation (CID) in the development of the triple-quadrupole mass spectrometer and on the importance and variety of mass-changing reactions in the overall field of tandem mass spectrometry.

Our own entry into mass spectrometry was in pursuit of the goal of creating a computer-controlled analytical instrument which would incorporate both separation and identification stages, and, in which the computer could randomly select the sample components to be identified. In the consideration of quadrupole mass analyzers for each stage of such an instrument, the immediate problem was how to energize the ions selected by the first quadrupole mass analyzer so that the second quadrupole would have a spectrum of  $m/z$  values to obtain for compound characterization. In a private conversation, Graham Cooks, who had already recognized the separation and identification processes inherent in mass-analyzed ion kinetic energy spectrometry (MIKES)<sup>1</sup>, encouraged us in this work and suggested that high-energy collision-induced dissociation (CID) might be easier than the electron-impact excitation that we were considering. High kinetic energy was considered necessary because, in the MIKES instruments, ion

<sup>1</sup>Cooks, R. Graham, "Collision Spectroscopy," R. Graham Cooks, Editor, Plenum Press, 1978, page 426

fragmentation efficiency was known to fall off dramatically below a few thousand electron volts of ion kinetic energy. In his later angle-resolved MIKES studies, Graham showed the fragmentation losses at lower energy to be due to the larger scattering angle of the products<sup>2</sup>.

The Office of Naval Research funded the tandem quadrupole project at Michigan State University (more for the novelty of the data system approach than the soundness of the mass spectrometry). The question of the method of interquadrupole ion excitation remained open at the time of the 1977 ASMS meeting (Washington, DC) when, in a serendipitous meeting with Jim Morrison of La Trobe University, Australia, we mutually recognized that the process that produced the background fragment ions interfering with his tandem quadrupole photon-induced fragmentation studies was exactly the process we needed for our proposed analytical instrument. Experiments performed by Yost on Morrison's instrument resulted in a publication demonstrating the feasibility of an analytical triple-quadrupole mass spectrometer<sup>3</sup> and in a patent on low-energy CID in an rf-only quadrupole on which Morrison is a coinventor. Simulations performed with the now-familiar (but then as yet unpublished) SIMION, developed by Morrison and McGilvery at LaTrobe, proved invaluable in these studies. Our results were confirmed by Hunt and Shabanowitz<sup>4</sup> on a triple quadrupole mass spectrometer they constructed in an incredibly short time, and by Cooks<sup>5</sup> on an equally spontaneous double-quadrupole mass spectrometer while the triple quadrupole in our laboratory was still under construction. Only a little later, our instrument produced data<sup>6</sup> and several studies on the energetics and utility of low-energy CID were performed in our lab and others<sup>7,8</sup>. Computer control and data acquisition for all the MS/MS scans were developed and one of the first instrument command languages for user programming of automated experimental sequences was developed<sup>9,10</sup>.

As a mass-changing reaction between mass analyzers, both high-energy and low-energy CID have great and undisputed power and usefulness. MS/MS with CID has been employed in a substantial fraction of the papers presented at this meeting. However, the field is far from mature. We are only approaching the adoption of a common format for MS/MS data and

<sup>2</sup>Laramee, J.A., P.H. Hemberger, and R.G. Cooks, *Int J. Mass Spectrom & Ion Proc*, **33** 231 (1980)

<sup>3</sup>Yost, R.A. and C.G. Enke, *J. Am Chem Soc*, **100**, 2274-2275 (1978)

<sup>4</sup>Hunt, D.F., J. Shabanowitz, and A. Giordani, *Anal Chem* **52**, 386 (1980)

<sup>5</sup>Zakett, D., R.G. Cooks, W.J. Fies, *Anal Chim Acta* **119**, 129 (1980)

<sup>6</sup>Yost, R.A. and C.G. Enke, *Anal Chem* **51**, 1251A (1979).

<sup>7</sup>Yost, R.A., D.C. McGilvery, D. Smith, J.D. Morrison, and C.G. Enke, *Int. J. of Mass Spectrom & Ion Proc* **30**, 127 (1979)

<sup>8</sup>Douglas, D.J., *J. Phys Chem*, **86**, 185 (1982)

<sup>9</sup>Newcome, B.H. and C.G. Enke *Rev Sci Instr*, **55**, 2017-22 (1984)

<sup>10</sup>Myerholtz, C.A., A.J. Shubert, M.J. Kristo, and C.G. Enke *Instruments and Computers*, **3** 13-14 (1985)

agreement on a universally useful experimental protocol still seems a long way off. There are few available libraries of MS/MS data and general rules for the structural interpretation of the full MS/MS data set (the product spectra of all significant precursors) are few. What is needed is the development of an expert system despite the lack of codified human expertise to implement. We have worked on a system to discover correlations between chemical substructures and patterns in the MS/MS spectra<sup>11</sup>, but a generally useful system has not yet been achieved. All these are areas where further development is needed to bring the applicability of MS/MS to the mature level enjoyed by practitioners of single-stage mass spectrometry.

The inherently low kinetic energy of ions in the triple quadrupole mass spectrometer makes it a natural environment for the observation of ion-molecule reactions. This is a form of mass-changing reaction in which the selected ion reacts with a neutral reactant gas introduced into the center quadrupole collision chamber. This can be called a collisionally-activated reaction (CAR) even though, in practice, the ion-molecule products are favored over the CID products at near-thermal kinetic energies. With our initial triple quadrupole instrument, we observed many interesting products, even with argon as the collision gas. With a deuterated selected ion or reactant molecule, a variety of H-D exchange products were observed. In 1981, Porter, Beynon and Ast<sup>12</sup> called the MS/MS instrument, "a complete chemical laboratory," in which synthesis, purification, reaction, and analysis occur in the source, MS1, collision cell, and MS2 respectively. This concept is still to be fully explored.

The CAR process can provide greater specificity than direct ion fragmentation. In Yost's lab, the cyclopentadiene molecular ion was readily distinguished from the linear 1-pentene-3-yne molecular ion (same  $m/z$  and composition) by CAR with isobutane while the CID products of these two ions differed only slightly in their relative ion abundances<sup>13</sup>. Yost's group also developed a "reverse" CAR system in which the effluent from a GC served as the collision gas. When piperidine molecular ion was selected by the first mass analyzer, the formation of product ions is selective for electrophiles (potential carcinogens)<sup>14</sup>. Orlando, Fenselau, and Cotter have demonstrated the efficient production of sequence ions of polypeptides by endothermic proton transfer reactions<sup>15</sup>. A scheme developed in Enke's lab for GC/MS/MS analysis of PCB's depends on the reaction of the selected molecular anion with O<sub>2</sub> reagent in the center quadrupole to produce the oxygen-chlorine

<sup>11</sup>for the most recent paper, see, Hart, K.J., P.T. Palmer, D.L. Diedrich, and C.G. Enke, *J. Am Soc Mass Spectrom.* 3, 159 (1992)

<sup>12</sup>Porter, C.J., J.H. Beynon, and T. Ast, *Organic Mass Spectrom.*, 16, 101 (1981)

<sup>13</sup>Fetterolf, D.D., and R.A. Yost, *Int. J. Mass Spectrom & Ion Proc.*, 62, 33 (1984)

<sup>14</sup>Freeman, J.A., J.V. Johnson, M.E. Hail, R.A. Yost and D.W. Kuehl, *J. Am Soc Mass Spectrom.*, 1, 110 (1990)

<sup>15</sup>Orlando, R., C. Fenselau, and R.J. Cotter, *J. Am. Soc. Mass Spectrom.* 2, 189 (1991)

exchange product ion<sup>16</sup>. By cycling through the appropriate precursor/product mass pairs as the chromatogram develops, a separate ion chromatogram can be obtained for each degree of PCB chlorination from 4 through 7. For the study of ion-molecule reactions, the ion trap and Fourier transform mass spectrometers have the advantages that the ion kinetic energy is inherently near-thermal and long and controlled reaction times are readily achieved. For incorporation into an analytical scheme, particularly for chromatographic detection, the triple quadrupole has the advantage that the process in each stage is continuous.

Even though mass-changing reactions have been stressed in this talk, charge-changing reactions between the mass analyzers are also effective. Charge-changing processes that have been used with MS/MS include charge stripping, charge inversion (Cooks), and neutralization-reionization (McLafferty).

Ion fragmentation has also been achieved by processes other than CID. Collision with a surface (SID) introduced in Graham Cooks' lab has been observed, extended, and applied by several others<sup>17</sup>. Another technique of growing significance is photon-induced dissociation (PID). Following early studies by Morrison and Vestal, many others have explored this technique<sup>18</sup>. High-efficiency PID in an interaction region between two time-of-flight mass analyzers has recently been demonstrated in Enke's lab<sup>19</sup>. Fragmentation efficiencies of 10% to 70% have been achieved in an instrument designed to produce ten or more PID product ion spectra per second so that MS/MS data sets can be obtained from chromatographically delivered sample.

The mass-changing reaction has quietly become the main thrust of MS/MS research following an early preoccupation with the hardware of mass analysis. From the examples cited, we can see that it is this process that is at the very heart of the ability of MS/MS to extend the characterizing power of single-stage mass spectrometry. The mass-changing reaction part of the MS/MS story is far from finished. For each of the techniques currently employed, still more work is needed to understand and characterize the fundamentals of the processes and thus apply them more effectively. Techniques for the improvement of the process efficiencies will be very welcome, and much work remains to be done in the development of more techniques that are selective for particular chemical structures or reactivity.

<sup>16</sup>Lopshire, R. J. Lee, T. Victorine, J. Bradford, and C. Enke, in preparation.

<sup>17</sup>For example, see works of Aberth, Mabud, Schey, and Wysocki

<sup>18</sup>For example, see works of Duncan, Reilly, Russell, Shlag, and Biemann

<sup>19</sup>Seeterlin, M., P. Vlasak, D. Beussman, R. McLane, and C. Enke, *J. Am Soc Mass Spectrom.* In press.