

Performance of the electrospray ion source during reversed-phase LC-MS analyses

Ioan Marginean, Ryan Kelly, Ronald Moore, David Prior, Brian L LaMarche, Keqi Tang, Richard D. Smith
Pacific Northwest National Laboratory, Richland, WA

Overview

In spite of the tremendous technological growth of LC-MS, the seemingly robust electrospray remains a relatively neglected and poorly understood intermediate. Changes in liquid composition during gradient elution LC-MS analyses are expected to affect the electrospray operation. Attempts to control the electrospray operating conditions (e.g., applied voltage or distance to the MS inlet) based on feedback from electrospray imaging¹ and – more recently – spray current measurements^{2,3} have been described; however, a clear understanding of the electrospray performance during gradient elution LC-MS measurements is still lacking. As such, we evaluated the performance of an electrospray ion source during reversed-phase LC-MS analyses.

Objectives

- Reliable electrospray current measurements during LC-MS analyses
- Feedback control of the electrospray ion source voltage during LC-MS analyses

Introduction

- LC-ESI-MS has become de facto standard for proteomics research
- The vast majority of LC-MS analyses are performed with little or no feedback with respect to electrospray performance.
- Direct optimization of the analyte signal prior to data acquisition is not possible, as for infusion measurements.
- Changes in electric field due to different emitter-MS inlet distance or different emitter length are generally not accounted for.
- The effect of changing liquid composition during gradient elution LC-MS analyses on the electrospray operation has not been evaluated.

Electrospray current measurements

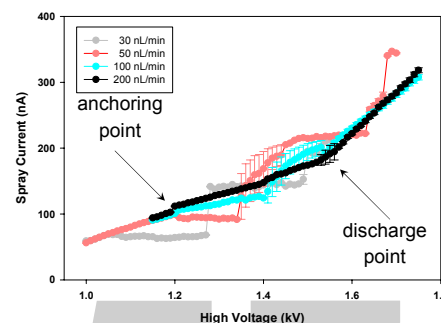


Figure 1. Electro spray characteristic curves⁴

The $I=f(V)$ curve at 200 nL/min loses the definition characteristic to lower flow rates:

- no cone-jet regime
- anchoring point
- discharge point

Emitter aging:

- unavoidable process accompanied by visual and operational clues
- accelerated by electrospray operation below the anchoring point or above the discharge point

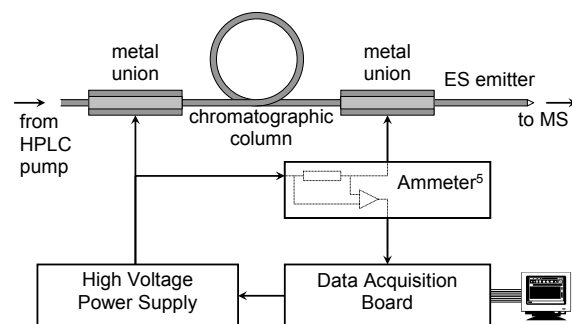


Figure 2. Instrument setup. Similar voltages are applied to both ends of the chromatographic column to minimize the effect of the leakage current on the spray current measurements.

Electrospray operation during LC-MS

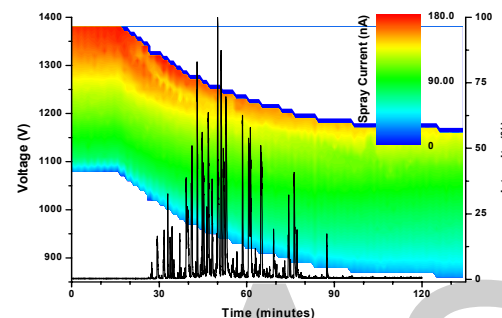


Figure 3. Gradient map⁶ composed of electrospray characteristic curves measured during a gradient LC-MS blank run. The base peak ion chromatogram (BSA tryptic digest) illustrates the time domain of analytical interest.

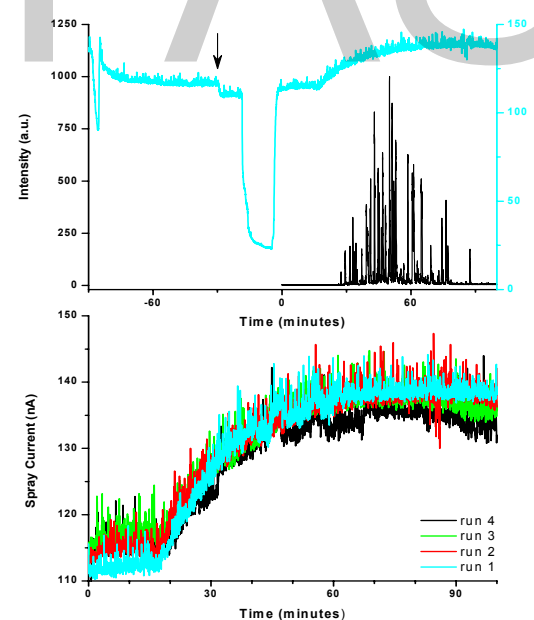


Figure 4. Base peak ion chromatogram and spray current for LC-MS analysis performed with constant (1250 V) electrospray voltage (top). Spray current traces for four consecutive LC-MS analyses (bottom).

Feedback control of the electrospray voltage

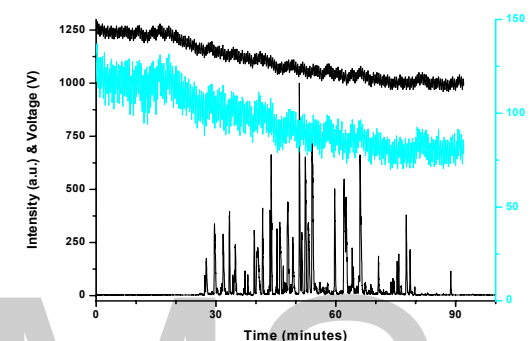


Figure 5. LC-MS analysis performed using feedback from spray current measurements to control the voltage with minimal *a priori* knowledge about the LC gradient.

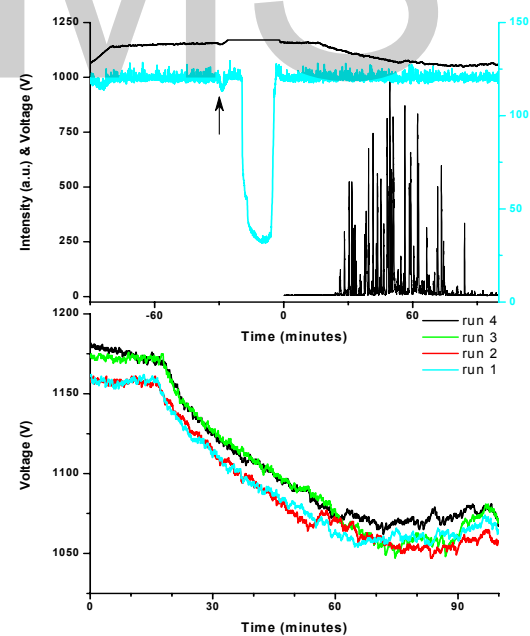


Figure 6. Base peak ion chromatogram, applied voltage and spray current for LC-MS analysis with feedback control of the applied voltage (top). Applied voltage traces for four consecutive LC-MS analyses (bottom).

Conclusions

- Spray current measurements can be used to monitor the electrospray operation throughout gradient LC-MS measurements
- Spray current measurements provide a reliable quality control mechanism and important information regarding electrospray operation by diagnosing emitter clogging, the presence of air bubbles in the eluent, and emitter aging
- Based on gradient maps, the electrospray voltage can be rationally selected to ensure optimal operation throughout the analysis
- Experimental conditions that bring more definition to the shape of the characteristic curves (different solvent systems, solvents of much lower conductivity, lower flow rates, flow split through multi-emitter arrays) could benefit significantly from more sophisticated voltage control algorithms

Acknowledgements

This work was funded by NIH National Center for Research Resources (RR018522). Samples were analyzed using capabilities developed under the support of the NIH National Center for Research Resources (RR18522) and the U.S. Department of Energy Biological and Environmental Research (DOE/BER).

Experimental portions of the work were performed in the Environmental Molecular Science Laboratory, a DOE/BER national scientific user facility at Pacific Northwest National Laboratory (PNNL) in Richland, Washington. PNNL is operated for the DOE by Battelle under contract DE-AC05-76RLO-1830.

References

1. Valaskovic, G. A.; Murphy, J. P.; Lee, M. S. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 1201–1215.
2. Staats, S. L. T.; Fogiel, A. J.; Suna, A. *Proceedings of the 56th ASMS Conference on Mass Spectrometry*, Denver, CO, June 1–5, **2008**.
3. Gapeev, A.; Berton, A.; Fabris, D. *Proceedings of the 56th ASMS Conference on Mass Spectrometry*, Denver, CO, June 1–5, **2008**.
4. Ioan Marginean, Ryan T. Kelly, Jason S. Page, Keqi Tang, Richard D. Smith. *Anal. Chem.* **2007**, *79*, 8030–8036.
5. Ioan Marginean, Ryan T. Kelly, David C. Prior, Brian L. LaMarche, Keqi Tang, Richard D. Smith. *Anal. Chem.* **2008**, *80*, 6573–6579.
6. Ioan Marginean, Ryan T. Kelly, Ronald J. Moore, David C. Prior, Brian L. LaMarche, Keqi Tang, Richard D. Smith. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 682–688.

CONTACT: Ioan Marginean, Ph.D.
Biological Sciences Division, K8-98
Pacific Northwest National Laboratory
P.O. Box 999, Richland, WA 99352
E-mail: ioan.marginean@pnl.gov