



# A New Ionization Source for Mass Spectrometry: Subambient Pressure Ionization with Nanoelectrospray (SPIN)

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*Significantly improving MS sensitivity by operating nano-electrosprays at subambient pressures*

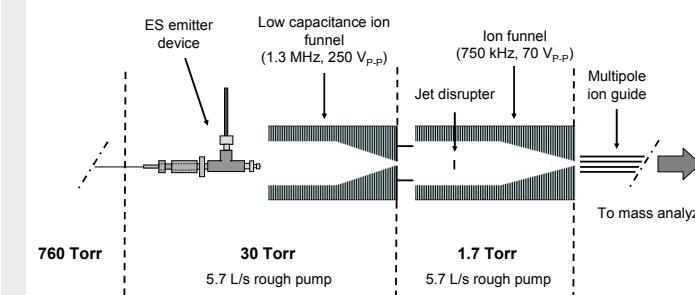
## Overview

- Large sample losses can occur in ESI-MS as the electrospray plume is sampled into the MS inlet capillary/orifice.<sup>1,2</sup>
- The inlet i.d. can be increased to gain ion sampling efficiency, but increased pumping requirements are needed, limiting routine use.<sup>3</sup>
- We eliminate these losses by removing the inlet and placing the electrospray source inside the mass spectrometer.
- The new source is characterized and evaluated using HPLC and demonstrates improved detection sensitivities.

## Introduction

- We present a new electrospray source design that can potentially eliminate ESI-MS interface ion losses.
- The new design locates the electrospray emitter in a reduced pressure region (~30 Torr) so that electrospray droplets are emitted directly into an electrodynamic ion funnel, eliminating the need for an atmospheric pressure inlet.<sup>4</sup>
- The new subambient pressure ionization with nanoelectrospray (SPIN) source allows the use of typical LC solvents, does not increase the interface pumping requirements, provides an effective desolvation region without electrical discharge, and improves MS sensitivity.
- We demonstrated stable electrosprays from 750 to 25 Torr. We also coupled the SPIN source to HPLC using gradient elution to analyze a protein tryptic digest sample, showing a 5- to 10-fold improvement in sensitivity versus a typical heated capillary inlet.
- Finally, we incorporated an electrospray emitter array in the SPIN source to further extend the sample flow rate range.

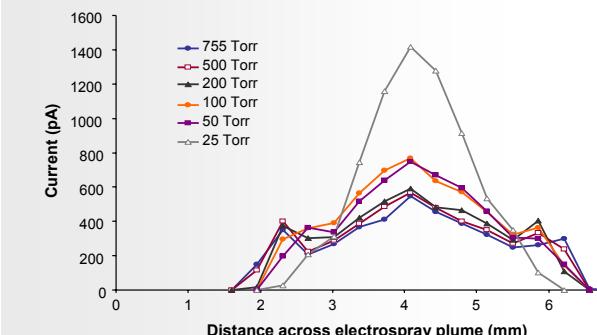
## Instrumentation



- The electrospray emitter assembly contains a 5  $\mu\text{m}$  i.d. chemically-etched emitter, counter electrode, and the capability of a sheath gas.
- The higher pressure ion funnel operates at 30 Torr and provides an effective desolvation region while focusing and transmitting the ions to the mass analyzer.
- The SPIN source and interface was installed on an Agilent single quadrupole mass spectrometer for characterization and testing.

## Testing and characterization

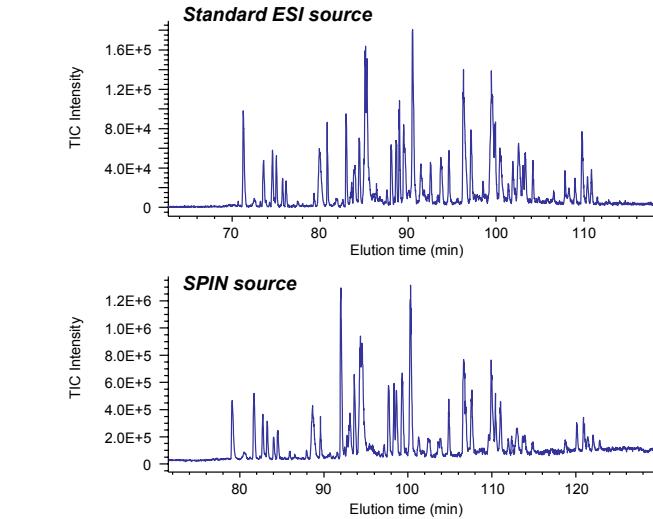
### Electrospray current profiles: ESI of a MeOH and H<sub>2</sub>O solution at different ionization chamber pressures



- Stable electrosprays achieved from 755 to 25 Torr
- Narrower electrospray plume at lower pressures caused by increased mobilities of the charged droplets
- Electrospray current remains unchanged through entire pressure range

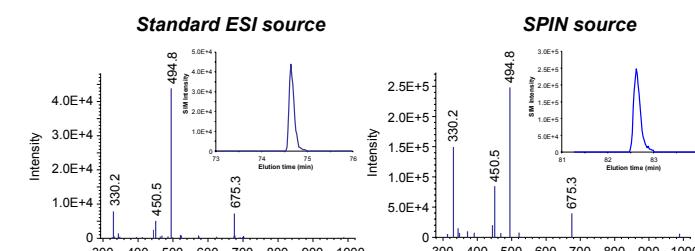
## Implementation with LC/MS

### LC/MS analysis of a protein tryptic digest solution



- 0.5  $\mu\text{g}$  of a BSA tryptic digest loaded onto a 75  $\mu\text{m}$  i.d. packed column with gradient elution (flow rate of 0.3  $\mu\text{L}/\text{min}$ )
- Standard ESI used a 7.6 cm long, 480  $\mu\text{m}$  i.d., heated capillary
- Both sources used 30 Torr and 1.7 Torr tandem ion funnels
- 5- to 10-fold sensitivity increase obtained with the SPIN source

### Mass spectra from the highest point in the elution profile of tryptic peptide m/z 494.8



- Similar mass profiles and increased peak intensities obtained with the SPIN source

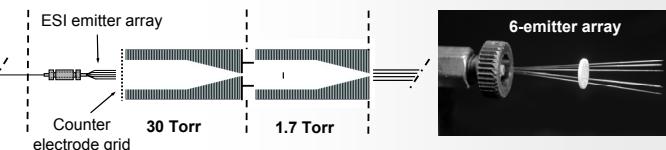
### Tryptic peptides from three replicate runs

m/z	charge state	sequence	LC/MS Peak		Standard ESI Source		SPIN Source		Intensity Increase
			average intensity $\times 10^3$	% stdev	average intensity $\times 10^3$	% stdev	average intensity $\times 10^3$	% stdev	
395.2	2	LVTDLTK	62	13.9	800	23.9	12.8	3.5	
424.2	2	LSQKPK	53	12.9	414	16.5	7.9	1.5	
464.3	2	YLYEIR	47	14.5	495	19.1	10.5	2.1	
480.7	3	RHPEAVSVLLR	49	23.8	486	3.5	9.8	1.9	
494.9	2	TPVSEKVT	37	19.0	193	24.7	5.2	1.3	
507.9	2	QTALVELLK	34	6.1	302	8.7	8.9	2.5	
547.5	3	KVPQVSTPTLVEVR	59	12.1	650	10.3	11.1	1.9	
571.9	2	QKTALVELLK	44	7.1	277	10.3	6.3	1.6	
655.3	3	LKPDPNLTCDFEKADEK	16	19.2	85	11.7	5.3	1.4	
735.7	4	AFDKEFLFTHAIDCTLPTEKQIKK	14	30.0	87	8.1	6.2	1.2	

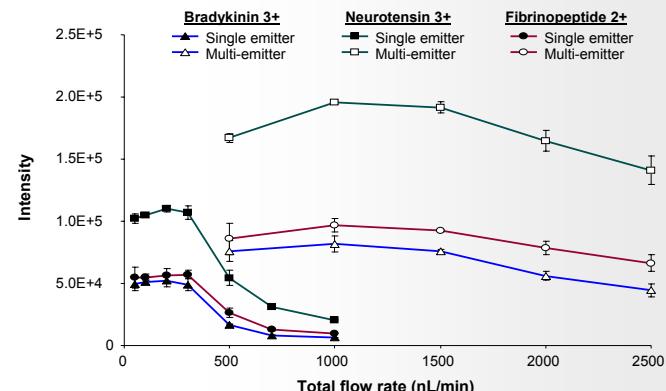
## Conclusions

- An electrospray ionization source has been combined with an ion funnel to allow ion production inside the first vacuum chamber of the mass spectrometer.
- The new ESI source eliminates essentially all ion loss associated with using an inlet to sample the electrospray current.
- HPLC was coupled to the new source to evaluate its use with proteomics analyses.
- Sensitivity increases of 5- to 10-fold indicate that ion transmission loss is greatly reduced while retaining the ionization efficiency.
- Use of an electrospray emitter array allows several individual electrosprays to be concurrently sampled, increasing the sample flow rate range.

## Using emitter arrays



### Infusion of a peptide solution at various flow rates using a single emitter and 6-emitter array with the SPIN source



- Peak intensities decline at higher flow rates with the single emitter due to incomplete desolvation
- Using an array of emitters reduces the flow rate per emitter, improving desolvation and allowing larger flow rates

## Acknowledgements

This research was supported by the U.S. Department of Energy (DOE) Office of Biological and Environmental Research, the NIH National Center for Research Resources (RR018522), the NIH National Cancer Institute (R21 CA126191), and the National Institute of Allergy and Infectious Diseases NIH/DHHS through interagency agreement Y1-AI-4894-01. Experimental portions of this research were performed in the Environmental Molecular Sciences Laboratory, a DOE national scientific user facility located at the Pacific Northwest National Laboratory (PNNL) in Richland, Washington. PNNL is a multiprogram national laboratory operated by Battelle for the DOE under Contract No. DE-AC05-76RLO 1830.

## References

- Cech, N. B.; Enke, C. G. *Mass Spectrom. Rev.* 2001, 20, 362-387.
- Page, J. S.; Kelly, R. T.; Tang, K.; Smith, R. D. *J. Am. Soc. Mass Spectrom.* 2007, 18, 1582-1590.
- Schneider, B. B.; Javaheri, H.; Covey, T. R. *Rapid Commun. Mass Spectrom.* 2006, 20, 1538-1544.
- Page, J. S.; Kelly, R. T.; Tang, K.; Smith, R. D. *Anal. Chem.* 2008, 80, 1800-1805.

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