

# Variable Flow Gradient LC-MS: Spray Mode Signal Affects and Control

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## Introduction

Variable- and switched-flow LC-MS/MS have proven to be useful tools for proteomics analysis of complex systems.<sup>1</sup> Such experiments are normally conducted under "pure" electrospray conditions at micro- and nanospray flow rates. It has been well established in aerosol science, that there are many possible and distinct spray modes under such conditions.<sup>2,3</sup> These modes are a function of flow rate, emitter design, and mobile phase composition. Furthermore, it has been established that such modes can affect the quality and character of the ion generation and subsequent MS and MS/MS spectra.<sup>4,5</sup>

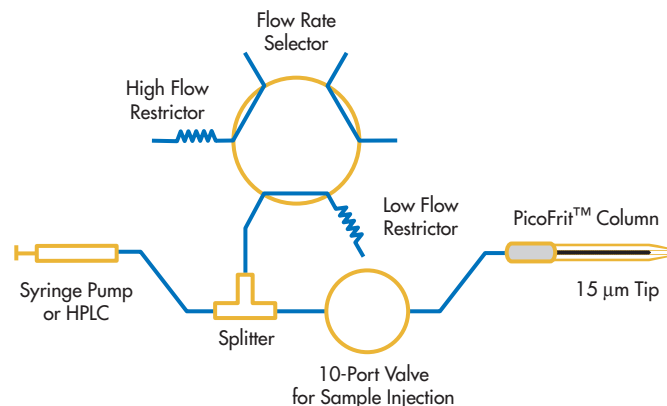
An unexplored area is the impact of such modes in a variable flow gradient system. Here we present both a continuous infusion experiment to investigate this behavior and an automated machine system for spray mode control.

## Methods

An ion trap mass spectrometer (LCQ™ Deca, Thermo Electron) was outfitted with a modified nanospray source (PicoView™ PV-500, New Objective, Inc.). The capillary inlet system of the ion trap was custom fitted with a gas tight stainless steel 2" capillary extension tube. This extension allowed the integration of an optimized illumination and spray visualization system.

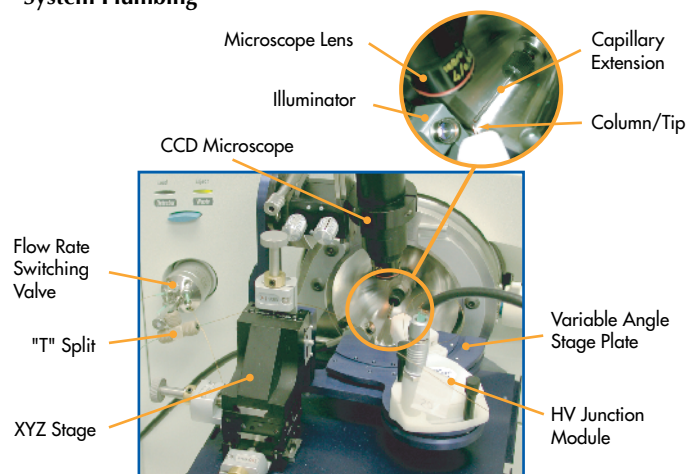
Continuous infusion experiments were conducted by delivering sample from a 500 nL gastight syringe via a syringe pump (PHD, Harvard Apparatus). A Tee-based flow splitter was placed between the pump and the nanospray source.<sup>1</sup> The side arm split of the Tee was fed to a two position switching valve (Valco). Each valve outlet was fitted with a 20 μm ID restriction capillary. When the nanospray source was fitted with a 75 μm x 10 cm nanobore C18 column having a 15 μm fritted tip (PicoFrit™, New Objective, Inc.) the restrictor lengths were adjusted to yield through column flow rates of 50 and 700 nL/min with the primary pump operating at a flow rate of 2.5 to 5 μL/min.

Five different angiotensin peptides (Michrom BioResources) were prepared in 10%, 30%, and 70% acetonitrile (0.1% formic acid) solutions at a concentration of 50 femtomole/microliter/peptide. For each mobile phase composition, full scan mass spectra were acquired continuously while switching the valve from the high to low flow positions every 30 sec. For each mobile phase data set, spectra were acquired at three different emitter (spray) potentials. Spectra were analyzed and summed for total ion and selected ion current.

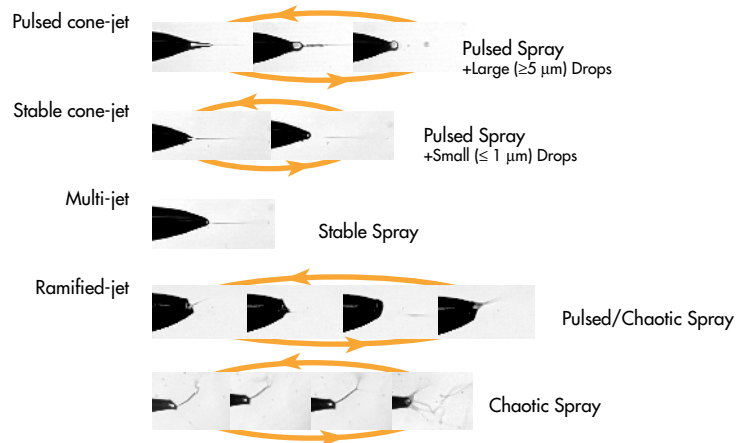


A 15 μm ID tip, normally recommended for a flow rate of 250 nL/min, was chosen to test the extremes of 50 nL/min (low flow) up to 700 nL/min (high flow).

## System Plumbing



## Spray Mode Analysis

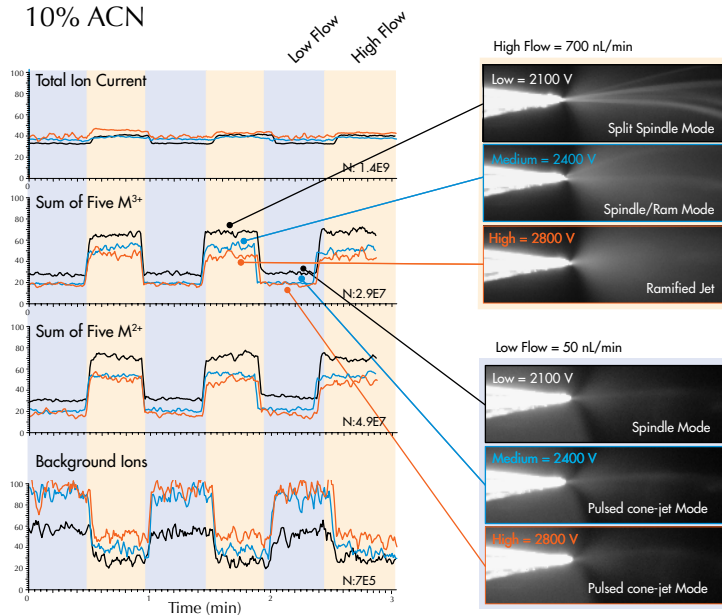


Transmitted light photomicrographs taken with a 1 μs pulsed strobe. 15 μm ID Tip

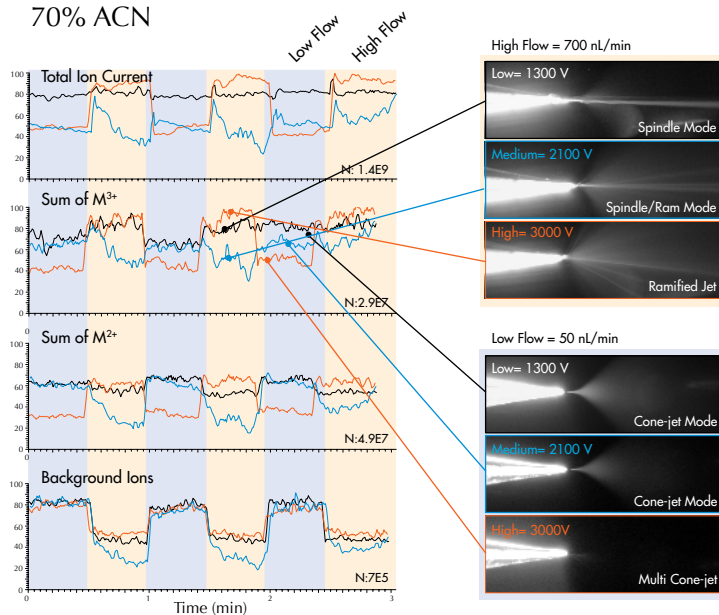
## Results

The following represent the total ion current and the summed ion current for a mixture of five angiotensins at 50 fmol/ $\mu$ L/peptide. The flow rate was switched from 50 nL/min to 100 nL/min every 30 seconds. The background ion is a summed ion current consisting of three ions.

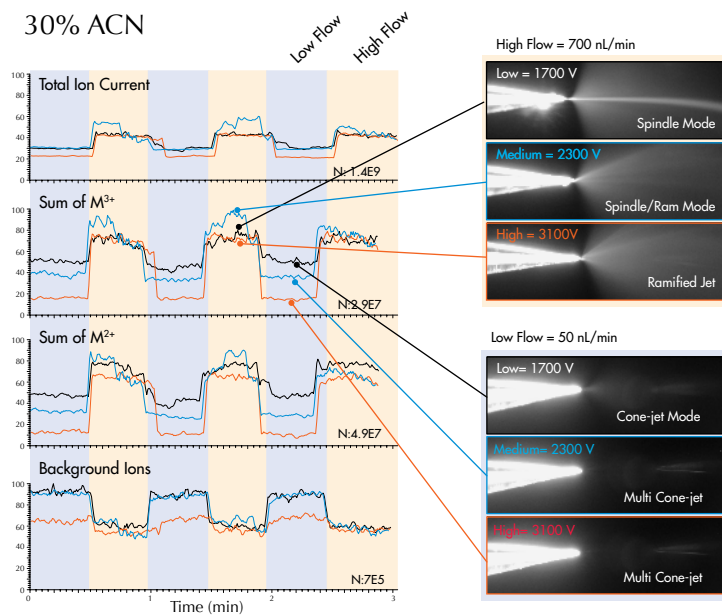
### 10% ACN



### 70% ACN

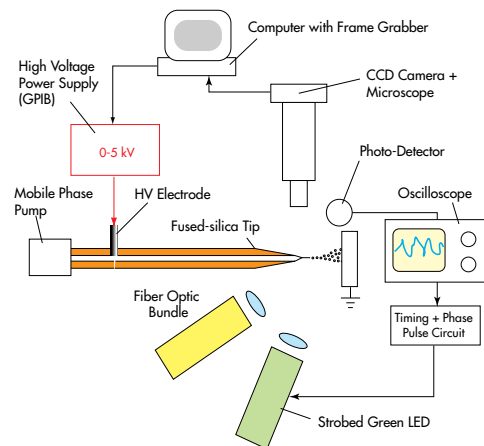


### 30% ACN

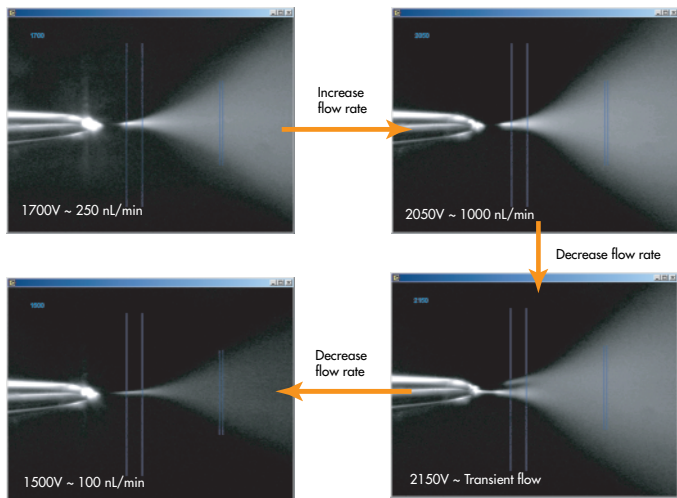


## Machine Vision Control

A machine vision based control system was based on the CCD camera microscope used in the nanospray source.<sup>6</sup> The output of the CCD camera was connected to a PC with a frame grabber, running LabVIEW™ 6.1 (National Instruments) for image acquisition. A 5 kV power supply (Stanford Research Systems Inc.) was controlled by the PC via GPIB interface bus. Reflected-scattered light illumination (150 W) was provided by a focused fiber optic bundle. A program to analyze the content of the CCD images based on the image morphology to automatically control the spray mode was written in LabVIEW. Mobile phase was delivered from a split flow capillary HPLC (Agilent).



The system can autonomously initiate spray and maintain a given spray mode for a variety of flow rates and mobile phase compositions. The self-correcting system varies applied voltage to maintain either the cone-jet or spindle spray modes.



A 30  $\mu\text{m}$  ID PicoTip™ was positioned 4 mm in front of the inlet. 70% Acetonitrile (0.1% formic acid) was delivered for a through-tip flow rate of 250 nL/min, ramped to 1  $\mu\text{L}/\text{min}$ , and then reduced to 100 nL/min over a 3 minute period. Using a 4 zone system, the control system could establish and maintain a stable spray. Unstable, multi-jet modes such as shown in the third photo above, persisted only a few seconds before the voltage was adjusted to re-establish a stable spray. Applied Voltage varied from 1300 to 2000 V during the run.

## Conclusions

- Switched flow control of a nanospray emitter can exhibit mass flow sensitivity, where total ion current is a function of flow rate. Such mass flow sensitivity can be minimized by observation of spray mode geometry and maintaining “desirable” spray modes.
- Mass flow sensitivity is minimized by:
  - Cone Jet Mode < Pulsed Cone-jet < Spindle < Multi-spray < Ramified jet
  - Proper match of tip size to (lower) flow rate
  - Operation of spray in spindle mode for high flow rate
  - Avoiding ramified jet and multi-jet modes
  - Higher organic content mobile phases
- A machine vision system is capable of maintaining desirable spray modes, and avoid those modes which decrease ion current, decrease baseline stability, or both.

## References

- [1] Martin, S. E.; Shabanowitz, J.; Hunt, D. F.; Marto, J. A. *Anal. Chem.* 2000, 72, 4266-4274.
- [2] Cloupeau, M.; Prunet-Foch, B. *J. Aerosol Sci.* 1994, 25, 1021-1036.
- [3] Jaworek, A.; Krupa, J. *Aerosol Sci.* 1999, 30, 873-893.
- [4] De Juan, L.; Fernandez De La Mora, J. *J. Colloid and Interface Sci.* 1997, 186, 280-293.
- [5] Juraschek, R.; Rollgen, F.W. *Int. J. Mass Spectrom.* 1998, 177, 1-15.
- [6] Valaskovic, G. A.; Murphy, J. P.; Lee, M. S. Proceedings for the 50th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando, Florida, June 2-6, 2002. Full Manuscript Submitted.