Automated Orthogonal Control System for Electrospray Ionization Mass Spectrometry

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Introduction

Low flow rate ESI (nanospray) demonstrates impressive figures of merit for sensitivity. When compared with conventional ESI (mL/min), however it suffers from a decreased robustness and reproducibility. Much of this behavior appears to be a result of the large number of spray modes possible at low flow rates.

The liquid and spray emitting for a tip may take on a wide variety of physical forms referred to as spray modes. The specific spray mode is highly dependent on the geometry of the emitter, the strength and shape of the electric field, flow rate, and the physiochemical characteristics of the mobile phase. These spray modes generate droplets of differing size and charge to mass ratio. Many modes are oscillatory, exhibiting pulsed droplet generation.

The most effective spray mode for producing droplets suitable for ESI-MS is the cone-jet spray mode. A stable, non-pulsing Taylor cone is formed, resulting in a stable ion current.

Optimization for this mode can be difficult with gradient chromatography because mobile phase characteristics such as surface tension, viscosity and flow rate change. One set of ESI tuning conditions is unlikely to yield a stable spray mode throughout an LC gradient. As a result compromises are often made, such as addition of sheath gas or operating at higher than optimal voltage. The resulting spray instabilities can shift charge state distribution and increase the relative standard deviation of ion current.

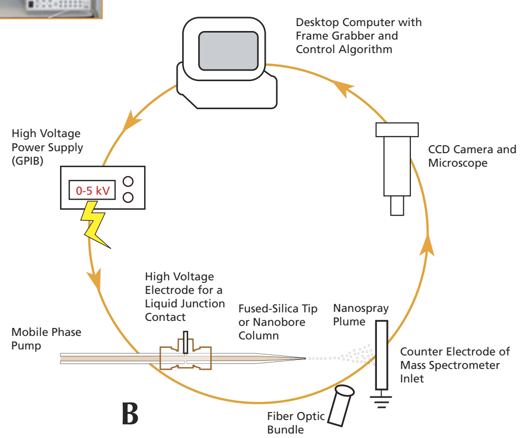
Methods

In an effort to make nanospray easy to use and more robust a self-tuning nano-ESI source system has been developed. The electrospray plume is directly monitored orthogonal to the mass spectrometer rather than the traditional ion current.

The implementation of a feedback loop results in a self-adjusting system (Figure 1). The system uses a video microscope equipped image acquisition computer to characterize the spray and determine the spray mode. A control algorithm then adjusts the applied voltage so that a desirable spray mode is obtained and maintained throughout the chromatographic analysis.

A

Figure 1 - (A) System configuration on an LCQ Deca™ (Thermo Electron) (B) Schematic of the spray analysis and orthogonal control system



B

A PicoView® nanospray source (New Objective) connected to an LCQ Deca™ (Thermo Electron) was used to test the self-tuning system. Voltage was provided to the source by a high-voltage power supply (Stanford Research Systems Inc.) controlled by a GPIB interface. A fiber optic bundle connected to a 150W tungsten-halogen illuminator provided intense illumination. A CCD camera based video microscope, positioned orthogonal to the ESI emitter, fed images into a PC equipped with a frame capture board. An image analysis and high voltage control algorithm was written in the LabView™ (National Instruments) programming environment. The electrospray voltage was under full control of the host PC and was completely uncoupled from a mass spectrometer.

Software

Spray mode determination is based on image morphology using edge detection and location within predefined areas surrounding the emitter called regions of interest (ROI) (Figure 2).

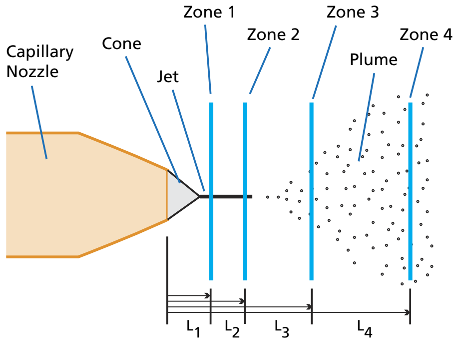


Figure 2 - Geometry of the four regions of interest (ROI) zones used by the image analysis algorithm

	Number of Edges			
Spray Modes	Zone 1	Zone 2	Zone 3	Zone 4
Dripping	2 or 4	2 or 4	2 or 4	2 or 4
Spindle	2	2	2	2
Pulsed Cone-jet	≥2	2	2	Ind
Stable Cone-jet	2	2	2	Ind
Multi-Jet	≥2	>2	>2	Ind
Multi-spindle	2 or 4	2 or 4	4	4

Table 1 - Analysis of the zones established in Figure 2 for different spray modes

A user friendly software platform was created to control the spray analysis algorithm. Screen captures of several steps from the program are displayed in Figures 3 - 5.

The software will recognize a nanospray emitter anywhere in the field of view of the camera (Figure 3). By locating the tip of the emitter, the ROI can be properly placed with a minimal amount of effort (Figure 4). Upon system activation a voltage ramping up procedure facilitates spray formation, eliminating any droplets which may have formed at the tip. The system continues to increase the applied voltage until edges area detected in zones one and two. At that point the spray mode analysis is activated (Figure 5). The detection of dripping or spindle spray modes cause the applied voltage to be raised; multi-jet or multi-spindle modes cause the voltage to be lowered. If a stable cone jet is detected the voltage is unchanged. A droplet detector alerts the user if liquid is collecting on the emitter.

Zone 1 - Probes the area corresponding to the cone-jet transition

Zone 2 - Positioned further away from the emitter probes the middle jet region

Zones 1 and 2 establish the number of jets or streams emitted

Zone 3 - Positioned along the leading area of the plume distinguishes between large droplets and plume formation

Zone 4 - Confirms the presence of large droplet emissions

Each spray mode has an identifying characteristic in each of these four regions. In one algorithm the mode can be determined by counting the number of edges in each ROI (Table 1).

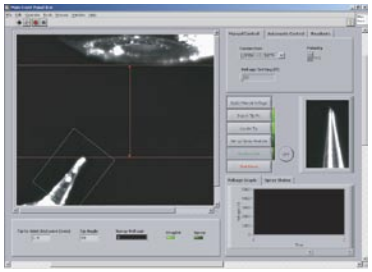


Figure 3 - The nanospray tip is automatically located and information such as tip distance and angle are displayed

Figure 4 - The ROI are defined by the user in the automatic controls tab

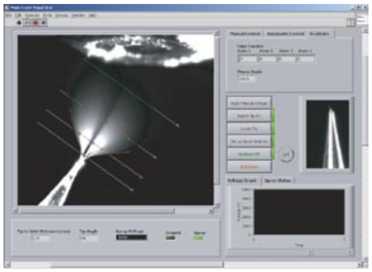


Figure 5 - Spray analysis of the multi-jet mode. The number of edges in each ROI and with the plume angle are shown in the readout tab

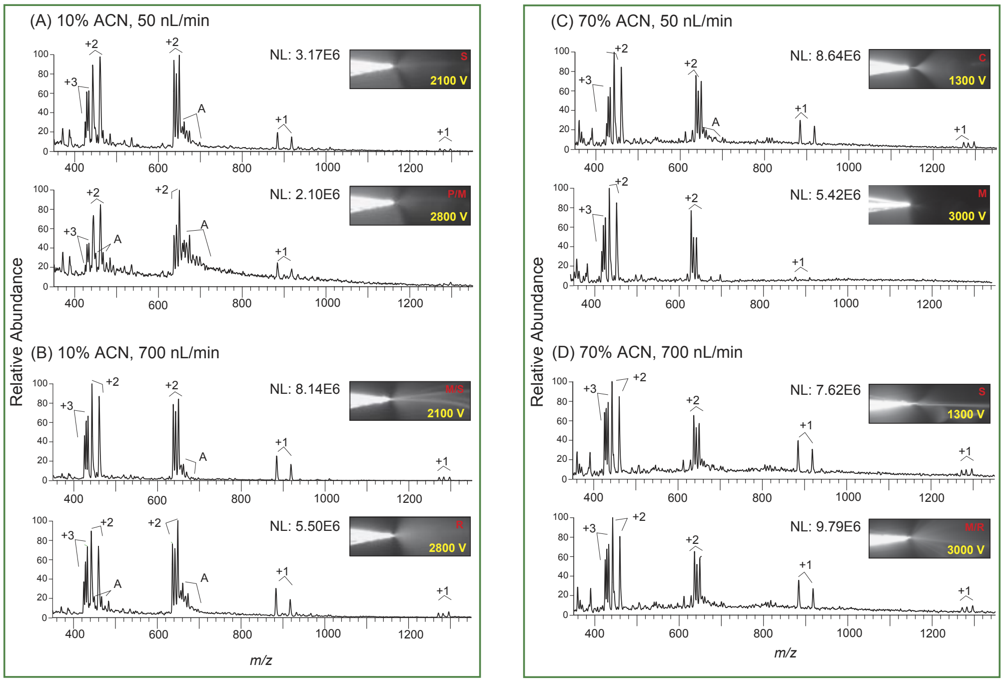


Figure 6 - Full scan mass spectra (summed, n=10) of a five peptide angiotensin mixture (50 femtomole/peptide/µL, 10% and 70% ACN). The spray image for each spectrum is shown in the inset. (A) Flow rate of 50 nL/min with emitter voltages of 2100 V and 2800 V. (B) Flow rate of 700 nL/min with emitter voltages of 2100 V and 2800 V. (C) Flow rate of 50 nL/min with emitter voltages of 1300 V and 3000 V. (D) Flow rate of 700 nL/min with emitter voltages of 1300 V and 3000 V. The letter in the upper right hand corner of the spray image indicates the spray mode observed (S=spindle, P/M= pulsed multi-jet, M/S=multi-spindle, R=ramified jet). The $(M+H)^{+1}$, $(M+2H)^{+2}$, and $(M+3H)^{+3}$ molecular ions are denoted with +1, +2, +3, respectively; A denotes adducts. The base peak ion intensity for each spectrum is shown to the left of each plume image. A 15 µm fritted tip was used.

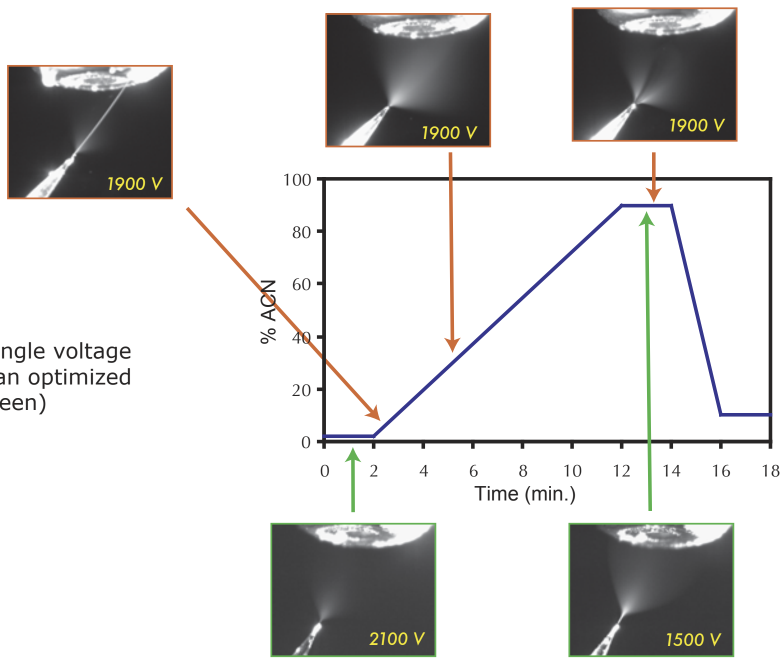
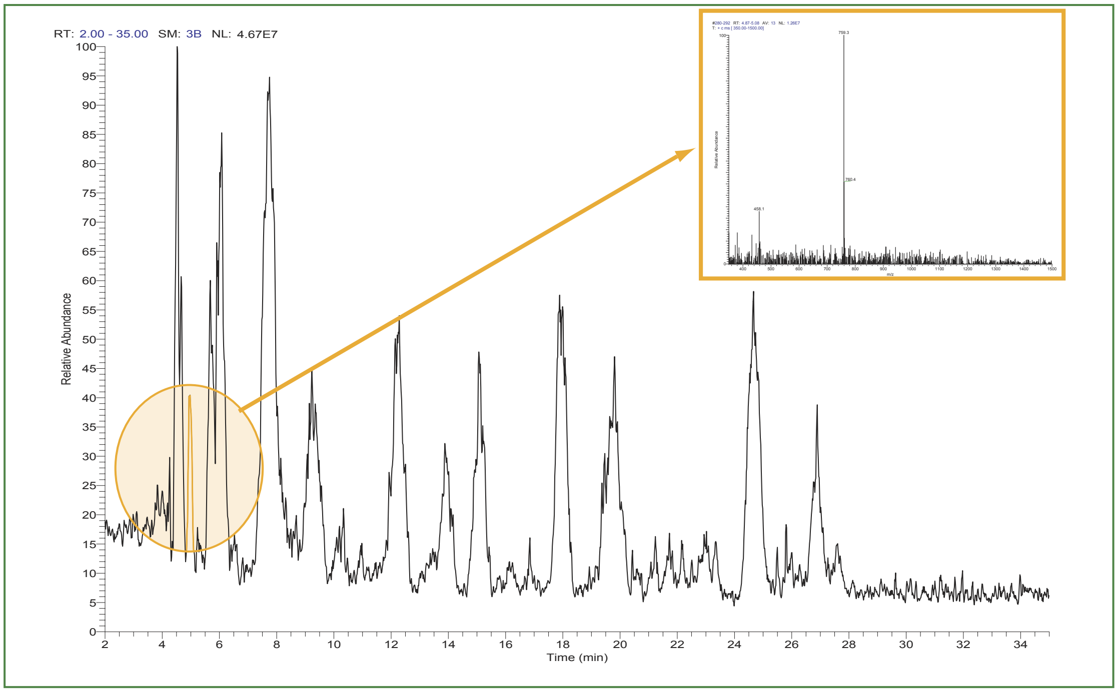


Figure 7 - Nanospray response to a single voltage across a gradient (top, orange), and an optimized voltage across a gradient (bottom, green)



1 µL Injection of a BSA digest (50 fmol/µL) using the automated control system across a 40-minute gradient. The optimal voltage ranged from 2350 V to 1900 V across the gradient, maintaining a stable and consistent spray. Inset: Full-scan MS, T=4.9-5.1 min. Note the high signal-to-noise for early eluting peptides.

Conclusions

- System operation on a nanospray equipped, ion-trap mass spectrometer has been demonstrated
- At or near the limit-of-detection optimal mass spectra correlate with particular spray modes (spindle and cone-jet preferred)
- The control system responds to changes in flow rate and/or mobile phase composition, maintaining optimal spray modes automatically
- Peak-parking experiments are readily optimized, providing both micro- and nanospray flow rates (see posters WPN-264 and ThPV-446 for more information)
- Control software automaticaly finds the tip, and guides user to proper set-up conditions (angle, distance) for improved repeatability
- Operation is unattended, enabling extended-time nanospray experiments

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