

An Automated Control System for Nano-Electrospray

James P. Murphy III and Gary A. Valaskovic
New Objective Inc, Woburn, MA

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 (Figure 1).

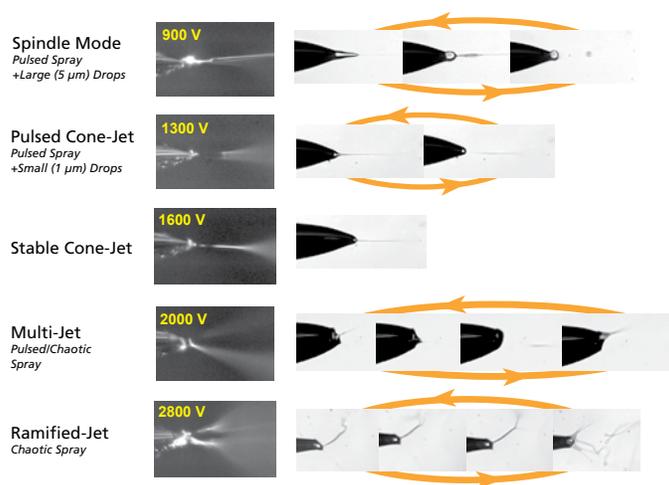


Figure 1 - Scattered light (left) and strobed, transmitted light (right) images for commonly observed spray modes. The applied voltage is on the upper left hand corner of the scattered light image. The emitter was 15 μm and the mobile phase was 50% MeOH, 1% acetic acid pumped at 250 nL/min.

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 2). 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.

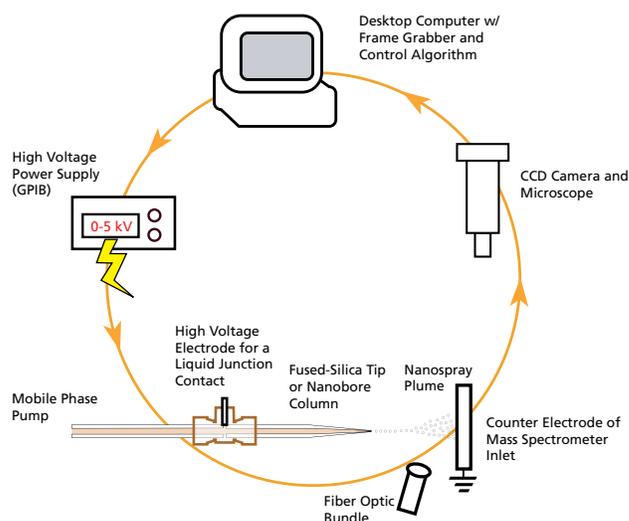


Figure 2 - Schematic of the spray analysis and orthogonal control system

System Components

A PicoView® nanospray source (New Objective) connected to an inlet shaped counter electrode 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 a LabView™ programming environment. The electrospray voltage was under full control of the host PC and will be completely uncoupled from a mass spectrometer.

The orthogonal illumination and imaging system generates signal for a feedback loop comprised of an analysis and control algorithm and adjusts the applied voltage so a particular spray mode can be obtained. The analysis is based on empirical image characterization and feature measurement. 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 3).

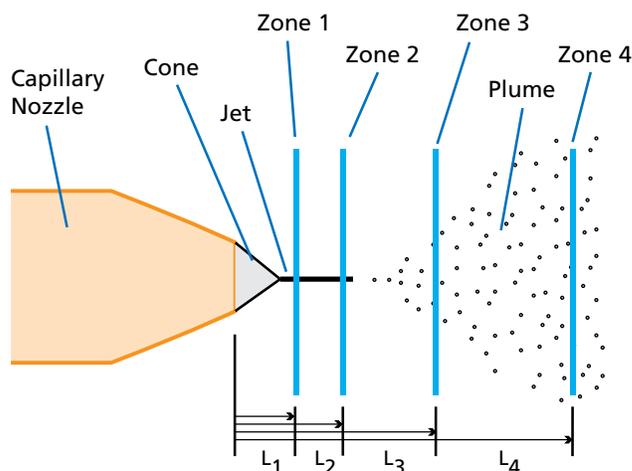


Figure 3 - Geometry of the four regions of interest (ROI) zones used by the image analysis algorithm.

- Zone 1 - Probes the area corresponding to the cone-jet transition
- Zone 2 - Positioned further away from the emitter probes the middle jet region
- 1 & 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).

Spray Modes	Number of Edges			
	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 - Zone analysis of the zones established in Figure 3 for different spray modes.

Software

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 4 - 6.

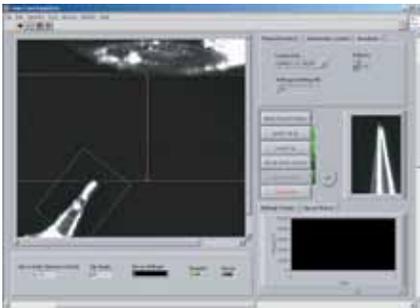


Figure 4 - The nanospray tip is automatically located and information such as tip distance and angle are displayed.

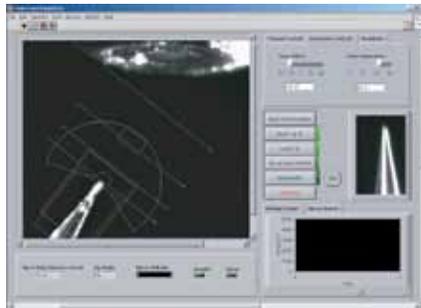


Figure 5 - The ROI are defined by the user in the automatic controls tab.

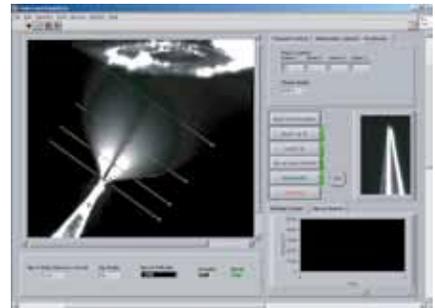


Figure 6 - Spray analysis of the multi-jet mode. The number of edges in each ROI and with the plume angle are shown in the readout tab.

The software will recognize a nanospray emitter anywhere in the field of view of the camera (Figure 4). By locating the tip of the emitter, the ROI can be properly placed with a minimal amount of effort (Figure 5). 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 6). 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.

Conclusions

The performance of the self-tuning system was tested with gradient chromatography. Figure 7 shows the typical results of a single voltage setting across a gradient and the response of the self tuning system across a gradient in which the applied voltage ranged from 2100 V at the high aqueous portion and to 1500V at the high organic portion.

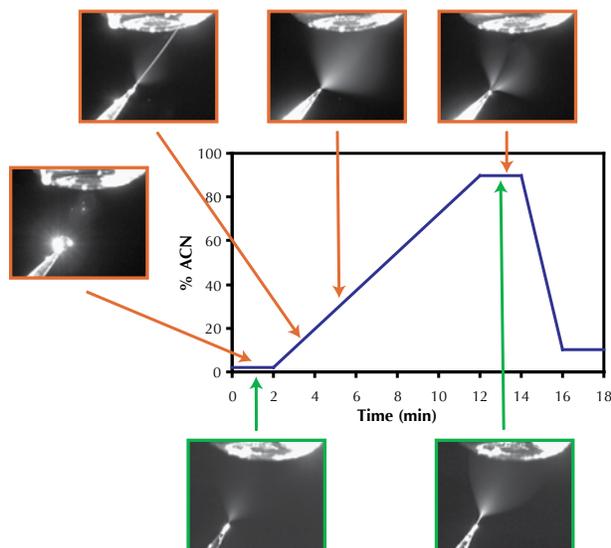


Figure 7 - Nanospray response to a single voltage across a gradient (top, orange), and an optimized voltage across a gradient (bottom, green).

In addition to gradient response, the system can adjust for changes in flow rate. The system requires little to no user intervention during operation, opening the opportunity for extended, unattended analytical experiments at nanospray flow rates.

System integration into a nanospray equipped mass spectrometer is currently under development.

Originally presented at the Association of Biomolecular Resource Facilities Conference, 2004

© 2004 New Objective, Inc. and Charles River Proteomic Services. All rights reserved. All trademarks and registered trademarks are properties of their respective companies.

