

Active Spray Control with Electric Field Optimization for Online NanoLC with Polymeric Spray Tips

S. L. T. Staats, A. J. Fogiel, Jr. and A. Suna, Phoenix S&T, Inc.

Overview

The primary obstacles to a successful nanoLC-MS campaign of hundreds of samples for protein profiling or biomarker discovery are clogging of the spray tips or columns, interrupted sprays due to the changes in the run buffer compositions in gradient LC, or air bubbles in the run buffers. Previous attempts to reinitiate spray after an intended spray stoppage include imaging the spray and digitizing the spray image, which would then be used to regulate the spray voltage or flow rates. A more effective way that is less sensitive to changes in the environment and easier to implement to ensure continuous spray is to monitor the spray current and use it as the input to the feedback loop, which controls the spray voltage/electric field by varying the distance between the nozzle opening and the mass spectrometer inlet.

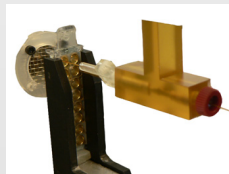
The experiment was carried out in an apparatus equipped with automated position optimization of the spray device for the mass spectrometer inlet. The spray devices used in this experiment were the polypropylene nozzle (20 μm i.d., 50 μm o.d.) and a 75 μm i.d. silica capillary capped with a porous polymeric plug and a mechanically tapered tip. These tips were chosen for their clog-resistance. The conventional laser-pulled silica capillary spray tips were also used for comparison. The spray current was obtained with an external current sensor placed in the vicinity of the mass spectrometer inlet (Thermo LCQ Advantage) and also internally with the spray current measured by and displayed through the data system.

The 16-well Nozzle Chip



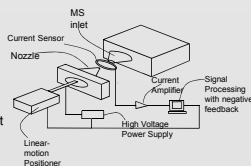
- Conical nozzle structures 0.5 mm to 3.5 mm in height
- 20+/- 3 μm i.d., 50 μm o.d.
- A reservoir of a few microliters to 15 microliters connects directly to each nozzle
- A collar with a 360 μm diameter forms a liquid-tight (up to 10,000 psi) pressure-fit junction with a capillary column
- 16 nozzles per chip; nozzles on 384-microliter plate spacing

Automated Nano-LC with spray current sensing



A nanoLC column (or just a capillary connected to a column downstream) is inserted into the nozzle robotically, and a high voltage for spraying is applied through a pin that makes contact with the gold coating covering the walls of the reservoir, or through a conducting liquid junction (union or Tee).

During nanospray, a small amount of the spray current is collected by the current sensor. When the collected current is less than a pre-set current threshold, the linear positioner moves the nozzle closer to the MS inlet to induce spray again. Likewise, a higher current threshold may be set to prevent the spray from becoming too large by moving the nozzle away from the MS inlet.



- The current sensing mechanism works in both the on-axis or off-axis positions.
- It is necessary to calibrate the current threshold settings because different buffers give different ion currents, e.g. the ion current from water is ~10x that of methanol.

Chip automation for Active Spray Control

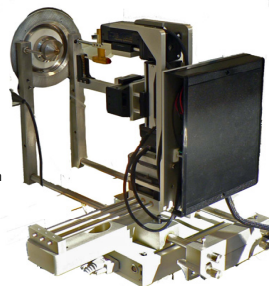
•3-D robotics for nozzle positioning +4th axis of motion for automated distance variation of the nozzle from the inlet

•Rotational stage for on-axis and off-axis spray positions

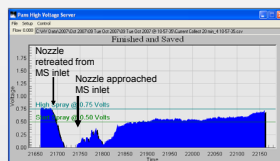
•NanoLC or Direct Infusion with the same interface

•High voltage application for LC is through a HV union and for direct infusion is through a glod coating close to spray tip

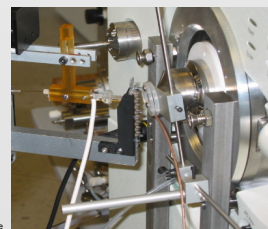
•Active spray control through negative spray current feedback



Negative Spray Current Feedback for Continuous Spray



The spray current trace collected by the current sensor. The feedback loop adjusted the nozzle distance from the MS inlet in order to keep the nozzle spraying continuously and the spray current intensity within the set window, in this case between 0.05 and 0.075 μA. The time constant of the feedback loop in the above trace was purposely set too slow to show the effects of the nozzle movements more clearly. The time unit for the x-axis was 50 ms/point.



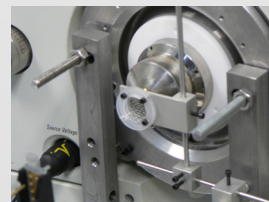
Active Spray Control for eliminating drips due to bubbles



- 1) Bubbles in the buffer lead to drips at the spray tip. Spray current is interrupted (in this case, dripping occurs).
- 2) Active spray control takes over and moves the nozzle close to the MS inlet to eliminate drips.
- 3) Once spray is resumed and the spray current is sensed, the active spray control system returns the nozzle to the original position.

Two current sensing mechanisms:

- 1) Current sensing wire mesh positioned in front of the mass spectrometer Inlet
- 2) Use of the inlet spray current measured by the mass spectrometer data system coupled with the external trigger menu



Experimental and Results

MS: LCQ Advantage

NanoLC: Micro-Tech XtremeSimple high pressure split-less pump

Column: 75 μm i.d., C18, 5 micron SureSpray column

Flow rate: 500 nL/minute splitless

Sample: The endorphin peptides were obtained from American Peptide and the bradykinin peptides from Sigma. The peptides were dissolved in 100 mM ammonium bicarbonate and diluted with 0.1% formic acid to ~1 pmol/μL.

Mobile Phases for LC:

A: H₂O + 0.1% Formic Acid
B: Methanol + 0.1% Formic Acid.

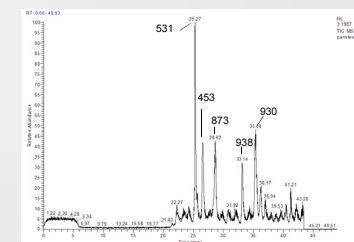
30 minutes of equilibration at 10% B

Gradient Program:

- 0-5 minutes 10% B (load step)
- 5-10 minutes 10-40% B
- 10-30 minutes 40-70% B
- 30-35 minutes 70-90% B
- 35-45 minutes 90% B

The peptide standards

Name	Mass	[MH+2] ²⁺
α-Endorphin	1744.82	872.4
γ-Endorphin	1857.90	930.0
Bradykinin1-9	1059.55	530.8
Bradykinin2-9	903.44	452.7



The mass chromatogram of the LC experiment with active spray control. The peptide standards were aged and showed some degradation products. The peak at 938 amu was that of the oxidized form of beta-endorphin, which exists even in fresh samples, but is more prominent here. The bubble/drip problem was not pronounced in this run, making the effects of active spray control less visible.

Summary

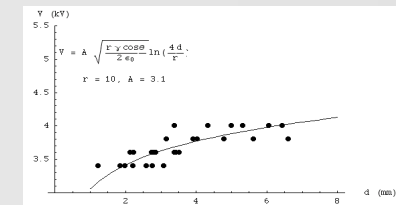
- A new plastic nanospray chip automated station with active spray control is presented.
- The active spray control based on negative spray current feedback ensures that the LC run is not disrupted because of spray stoppage due to changes in buffer compositions in gradient LC or air bubbles in the solvents. Spray is stable from 95% aqueous to 95% organic.
- An analysis based on the established equation for electrospray shows that the pronounced dependence of the spray onset voltage on the distance between nozzle and MS inlet is not due to a new spray phenomenon for the plastic nozzle or another polymeric spray tip. The open-ended laser-pulled capillaries do not show as pronounced a distance-voltage dependence.

Acknowledgements: Phoenix S&T, Inc. wishes to thank the NIH for partial funding support for this work through a Small Business Innovative Research grant award. We also thank Diane Cripps for assistance in the chromatographic separations of the peptides.

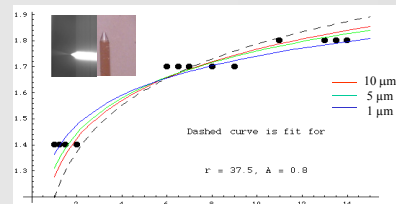
C. Voltage-Distance Dependence Analysis

Nozzle diameter ~20 μm, Best nozzle diameter to fit data ~20 μm using the well-known equation for electrospray below.¹

No new theory was needed for describing V-D dependence.



The prefactor of 3.1 was obtained from geometric factors such as the conical angle of the microfluidic channel leading to the nozzle where the Taylor cone formation could initiate and also the estimated contact angle of the methanol/water mixture. The scatter in the data may have been due to the surface unevenness of the plastic inside the microfluidic channel. The agreement of the data to the established electrospray equation came as a surprise since the plastic nozzle is very different in both structure and electrical conductivity from the conducting tubular structure that was used to construct the model that results in the equation in reference 1.



Another polymeric sprayer, a tapered silica capillary (360 μm o.d., 75 μm i.d.) with a polymeric sol-gel porous frit was also used for the distance-voltage measurements. The results also show that the distance-voltage relationship agrees with the established theory except that the apparent tip diameter was considerably smaller than the 75 μm (<20 μm) of the silica capillary, indicating that the Taylor cone that formed extended over only a portion of the frit. It might be that a small droplet exuded from the pores of the frit first, then turned into the Taylor cone. Interestingly, a conventional laser-pulled capillary tip did not show as pronounced a voltage-distance dependence as did the polymeric spray sources.

References:

¹P. Kebarle and L. Tang. Anal. Chem. **65**, 972, (1993)