Using Electrical Current to Monitor Nanospray During an LC Gradient for Robust and Reproducible High Throughput Nano-Liquid Chromatography

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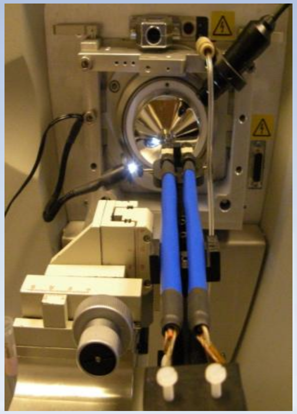
Overview

With the increasing need to detect sub-femtomole concentrations in complex samples, the preferred mass spectrometric technique for biological samples has become nanospray due to its increased sensitivity (10x of conventional ESI) and minute sample consumption. However common occurrences such as emitter clogging and dripping make it difficult to use in high throughput applications. The loss of spray, even for a few seconds, could be detrimental to data acquisition for both data base searches and quantitation.

The work presented here shows that not only the spray current but also the total current of the system including the current due to the buffer in the transfer line may be exploited to be an indicator of the modes of spray, such as cone jet, pulsating, and the loss of spray, and presents a novel current feedback mechanism for the detection of and the recovery from the loss of spray through automated responses such as moving the spray emitter closer to the mass spectrometer inlet to increase the electric field and to remove any droplets so as to reduce the loss of data. The use of the current as an indicator that the gradient pump is working properly is also discussed.

µAutoNano

All experiments were performed with the µAutoNano, an automated dual column switching nanospray source (*Phoenix S&T, PA*).



• Spray restoration when switching columns is assisted by a nitrogen purge and a 'wipe' function in which the nano-LC column is moved closer to the mass spectrometer and then is moved back to the original position. These responses are triggered by a negative feedback loop monitored by the current.

• High voltage from a +/-8KV power supply is applied to a metal union that connects the LC transfer line to the column

• The unit has a camera and light source for visualizing the spray.

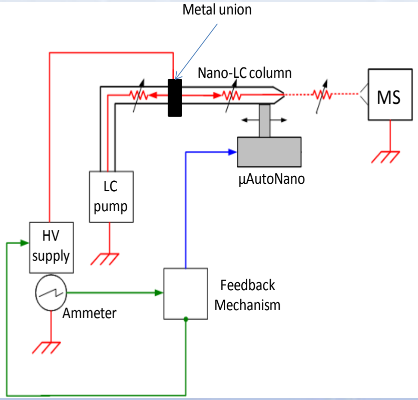
Methods

• The following sprays were produced with a pulled but truncated, fritted 75µm I.D. column packed with 9 cm of Exsil C18 3µm packing material (*Alltech*)
Mass Spectrometer

• All experiments were performed using the MicroTech splitless high pressure nanoflow pump using Buffer A (99.9% H_2O , 0.1% Formic Acid) and Buffer B (99.9% ACN, 0.1% Formic Acid).

• All mass spectrometry data was collected with a Thermo LCQ Advantage Mass Spectrometer

Feedback Mechanism for Restoring Lost Spray



• Buffer was supplied with a grounded nanoLC pump.

• Nanospray was performed by applying high voltage to the metal union and buffer was sprayed directly into the mass spectrometer

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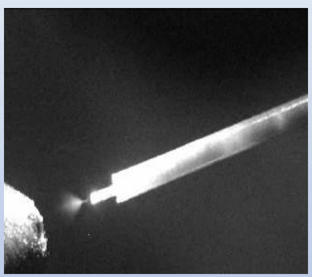
• The current (in the red track) was measured with an internal ammeter in the power supply. The measured current includes current of the buffer going back to the LC pump as well as the current of the spray

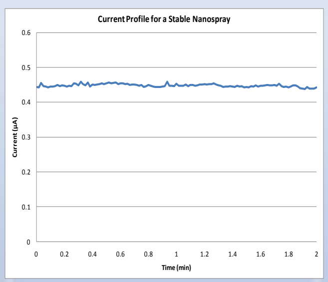
• The feedback mechanism was based on comparing two current values, T0 and T1, at two consecutive time intervals. If T1, the newer measured current value, was lower than T0 by a specified percentage, a loss of spray had occurred. A signal was sent to the µAutoNano to move the emitter closer to the inlet to restore the lost spray. The time intervals for the T0 and T1 measurements could be adjusted by the user.

Results

Types of Nanospray

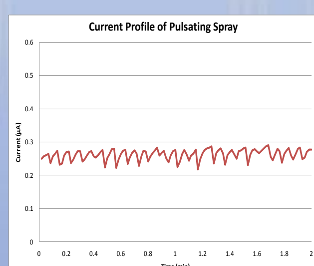
The buffer sprayed was 50/50 A/B at 300nL/min.



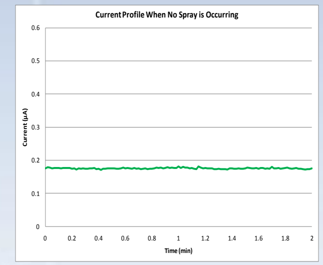


• The voltage for the stable spray was kept at 2.3kV

• The current remained consistent through out with an average of 0.45µA and an RSD of 1%



• The voltage was lowered to 2.0kV to produce a pulsating spray
• Each dip in the current corresponded to a drop being emitted followed by a build up of another drop.
• The averaged current remained constant at 0.52µA with an RSD of 1%

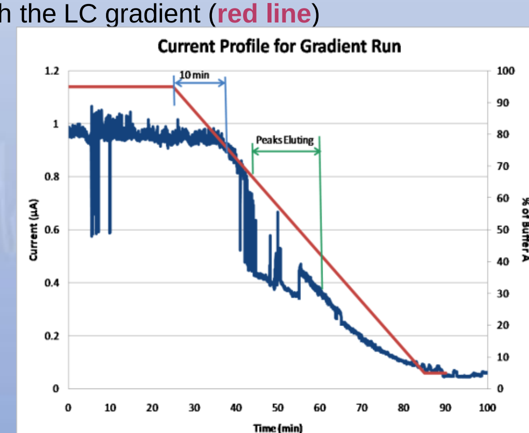


• The emitter was removed from the inlet to demonstrate the drop in current when no spray was observed.
• The average remained consistent with a value of 0.18µA with an RSD of 1% and this was 60% lower than when it was spraying

Current for a Gradient With Bovine Serum Albumin Digest

300 fmol of BSA digest was loaded onto the column

The following graph shows the current profile of the gradient (**blue line**) plotted with the LC gradient (**red line**)



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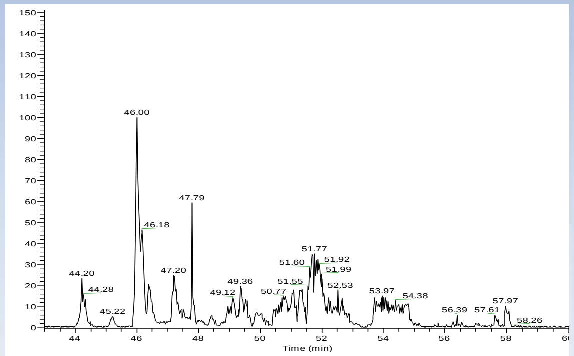
Chromatogram of 300fmol BSA digest



• The current decreased as the concentration of acetonitrile increased

•Since the voltage was not optimized for all buffer concentrations, the dips in the current were indicative of disturbances in the spray. Most of the disturbances that were observed occurred during the elution of the analyte.

•The 10min shift indicated in the graph represented the delay time for the start of the gradient, i.e., the pump program was erroneously off by 10 minutes.

Spray Restoration Using the Feedback Mechanism

The buffer sprayed was 95/5 A/B
The voltage was maintained at 2.5kV

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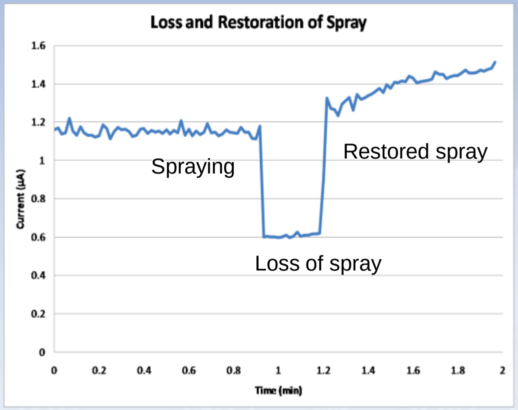

• Spray loss is shown with a 49% drop in current

• After 3s of the spray not returning, the µAutoNano automatically moved the emitter toward the MS inlet to restore spray within 14s of spray loss. This response time can be adjusted by the user.

Summary and Discussions

This work has shown the benefits of using the current feedback system for a variety of applications including drug discovery and biomarker research. The benefits that were found within this work include:

•The power supply measures the total current which includes the current due to the electrical conductivity of the buffer in the transfer line from the LC pump to the spray tip , where the spray current returns to electrical ground via the mass spectrometer. At 50/50 A/B, the current that is only the spray portion contributes 60%.

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•The current indicates the types of spray observed for nanospray. A more stable current represents a stable cone-jet mode with a Taylor cone, and a pulsating spray shows cyclic dips in the current.

•With a gradient run for a sample, the current decreases as the concentration of the organic component increases. The acetonitrile has a lower electrical conductance than water, therefore causing the current to be lower than that for a higher aqueous buffer concentration. This feature could also be used as a diagnostic tool for the LC pump functions. With the current, we were able to determine that there was an unexpected 10min delay by the pump in the start of the gradient. Other pump malfunctions such as missed injections and the loss of one side of the binary pump were also detected in the total current.

•A feedback mechanism is designed to distinguish between when peaks are eluting vs. when spray is lost from the emitter, and then to restore the lost spray by automatically moving the emitter closer to the mass spectrometer inlet. Spray can be returned in seconds.

Thus the total current feedback mechanism can not only provide continuous robust nanospray that prevents the loss of precious data for biological samples, but also be used for pump diagnostics.