

# Inline Electropray Current Conductivity Detection for Characterizing Mobile Phase Composition and Gradient Delay

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## Introduction

Nanobore liquid chromatography-mass spectrometry (nLC-MS) is the favored platform for peptide analysis and protein identification. The application utilizes a packed-tip approach where column effluent sprays directly off the column outlet. Due to introduction of undesirable post-column volume, in-line optical (ultraviolet) detection is eliminated as a viable method. There is often a need, especially with split-flow pumps, to independently characterize fundamental nLC operating parameters such as gradient delay time (from pump to column), real-time mobile phase composition at the head of the column, and through-column flow rate. We herein employ a novel conductivity "cell", based on a method originally developed for electroosmotic flow-rate characterization in capillary electrophoresis<sup>1</sup>, as an effective inline conductivity detector to independently measure gradient delay and mobile phase compositional changes at the column inlet. Two separate true-zero-dead-volume (ZDV) electrically conductive unions, separated by a 100 to 230 nL swept volume, were configured to enable mobile-phase conductivity measurement. This method adds no post-column volume, does not adversely impact chromatographic quality, and provides a straightforward means of determining some basic chromatographic operating parameters.

## Methods & Materials

### Instrumentation & Components:

- Ion-trap mass spectrometer (LCQ™ Deca, Thermo Fischer Scientific)
- Customized nanospray source (Digital PicoView® 150, New Objective)
- NanoLC pump (Eksigent)
  - Mobile phase A: 0.1% formic acid in HPLC-grade water
  - Mobile phase B: 0.1% formic acid in HPLC-grade acetonitrile
- PicoFrit® packed-tip emitters (New Objective)
- Keithley Picoammeter
- Fluke microammeter with RS-232 communication port
- PicoClear™ Conductive Unions (New Objective)

### Set-up:

Using two electrically conductive, transparent, ZDV unions, voltage was applied, and a grounding locus was defined in the flow path. Each union contains a 150 µm diameter platinum wire electrode separated by 5 cm of 20, 50, or 75 µm-ID fused-silica tubing. The union closest to the column was connected to the high-voltage electropray power supply from the mass spectrometer. The other union was connected to a PC-interfaced microammeter providing a virtual electrical ground. A 10 cm packed column containing a 75 µm tubing ID and 15 µm tip ID was employed in the analysis. Samples were injected via a 10-port automatic nano-valve from a 0.5, 1.0, or 5 µL loop. All current data were transmitted to a PC via an RS-232 serial port.

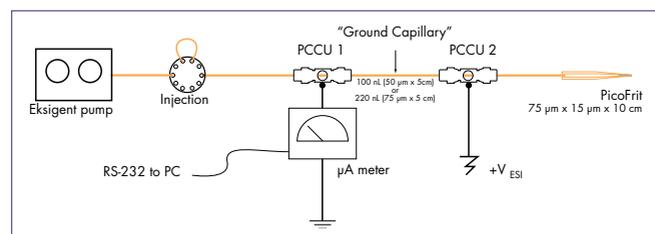


Figure 1 Configuration schematic

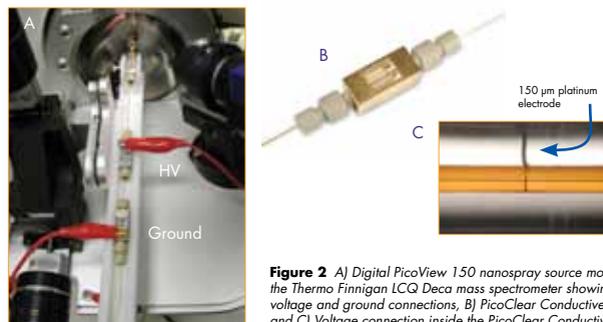


Figure 2 A) Digital PicoView 150 nanospray source mounted on the Thermo Finnigan LCQ Deca mass spectrometer showing high-voltage and ground connections, B) PicoClear Conductive Union, and C) Voltage connection inside the PicoClear Conductive Union

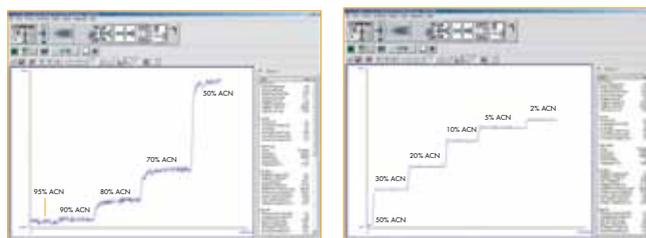


Figure 3A Calibration profile of current vs % ACN for a 75 µm x 5 cm ground capillary. Nanospray source current was plotted against time. ESI voltage was 1.8 kV, flow rate of 500 nL/min.

Figure 3B Calibration profile of current vs % ACN for a 75 µm x 5 cm ground capillary. For compositions less than 50% ACN. ESI voltage was 1.8 kV.

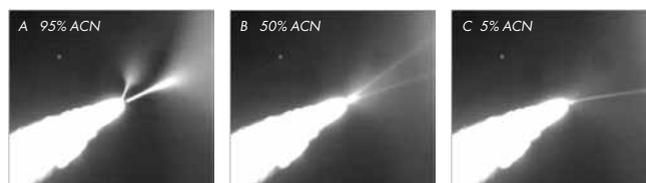


Figure 4 ESI plume photos at 95%, 50%, and 5% ACN, respectively. The flow rate was 500 nL/min. 15 µm PicoFrit emitter with an applied voltage of 1.8 kV.

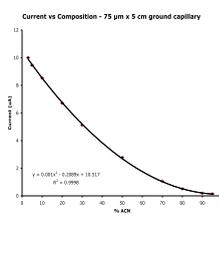


Figure 5 Ground current vs % ACN for a 75 µm x 5 cm ground capillary using the data from Figure 3

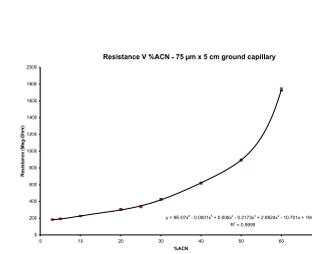


Figure 6 The electrical resistance in MΩ vs % ACN for the 75 µm x 5 cm ground capillary. A high-impedance MΩ meter with a 2000 MΩ upper limit was used to measure the resistance. Mobile phase compositions > 60% ACN displayed resistance exceeding 2000 MΩ.

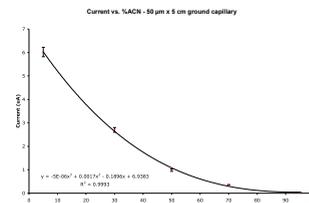


Figure 7 Ground current vs %ACN for a 50 µm x 5 cm ground capillary. The electropray voltage was 2 kV.

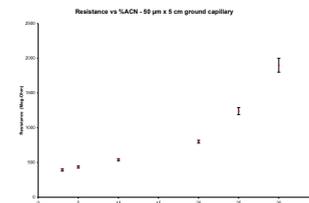


Figure 8 The electrical resistance in MΩ vs %ACN for the 50 µm x 5 cm ground capillary. A MΩ meter with a 2000 MΩ upper limit was used. Mobile phase compositions of greater than 20% ACN had a resistance of more than 2000 meg-ohm.

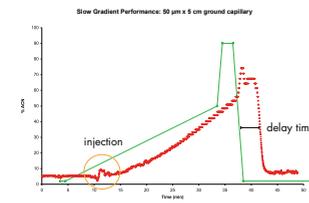


Figure 13 Calibrated %ACN vs. time obtained using the calibration curve from Figure 7. The programmed gradient is shown in green. Note: Because the µA meter is not sensitive above 70% ACN, the calibrated plot reaches a saturated value near 70%. The 0.5 µL injection plug is evident near the beginning of the run.

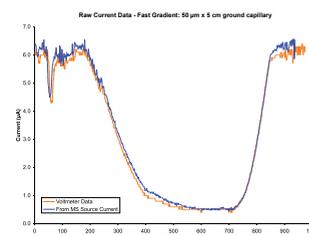


Figure 9 Raw, unfiltered current traces obtained for a fast gradient at 500 nL/min. The electropray voltage was 2 kV. The voltmeter data (orange) and the source power supply current (blue) show good agreement, indicating only a small portion of the source current is required to maintain the nanospray plume.

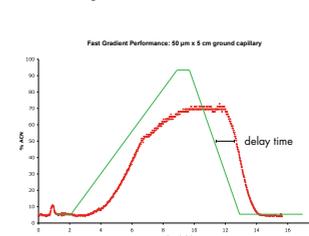


Figure 10 Application of the calibration curve (Figure 7) to the current trace (Figure 9) yields a plot of % ACN vs time. The programmed gradient is shown in green. Note because the µA meter is not sensitive above 70% ACN, the calibrated plot reaches a saturated value near 70%.

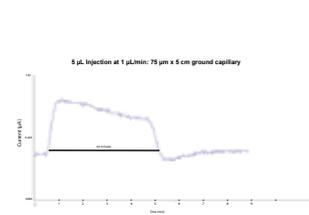


Figure 11A A current trace for an injection plug from a 5 µL injection valve. A sample of two angiotensin peptides at 1 µM concentration in 5% ACN was injected into a flow stream containing 30% ACN at a 1 µL/min. flow rate.

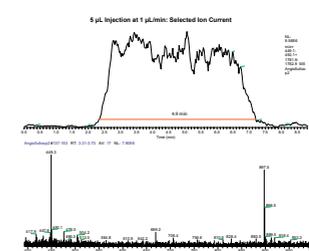


Figure 11B Mass spectral acquisition of the injection profile from Figure 11A. Note the current monitor provides a similar time profile, suggesting the current monitor may be useful in monitoring on-column sample injection.

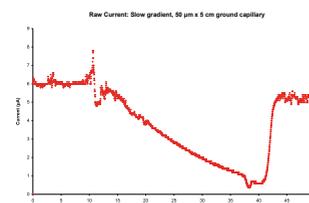


Figure 12 Raw, unfiltered current trace obtained for a traditional slow gradient at 300 nL/min from a 50 µm x 5 cm ground capillary. The electropray voltage was 2 kV. The gradient-programmed profile is shown in Figure 13.

## Results

### Operating Principle:

Electrical resistance of the mobile phase within a given section of capillary tube is a function of mobile phase composition. Due to efficient proton transfer, highly aqueous mobile phase (0.1% formic acid) exhibits relatively low electrical resistance. As the mobile phase becomes richer in acetonitrile, an aprotic co-solvent, the electrical resistance rises considerably. The ground point located upstream from the high-voltage contact point provides for current flow between the two points, following Ohm's Law. Provided the following occur, stable electropray is maintained:

- (1) The ground current does not exceed current output capacity of the high-voltage supply
- (2) Electrical resistance between the high-voltage contact and emitter orifice is less than the electrical resistance at which the grounding capillary is maintained (preferably much less)

## Conclusions:

- Provided the resistance in the ground capillary is not too low relative to spray capillary resistance, a grounding union may be inserted upstream of the high-voltage contact point without disturbing spray stability
- With an "empty" spray emitter, a 75 µm x 5 cm ground capillary provides good spray stability and a full-scale ground current range of 10-14 µA at 5% ACN, 0.1% formic acid
- With a packed-tip emitter, a 50 µm x 5 cm ground capillary was required for a stable spray, yielding a full-scale ground current range of 3-6 µA (5% ACN).

Note: The 75 µm x 5 cm capillary proved unsuitable, yielding poor spray stability.

- The current vs %ACN relationship is nonlinear, based on the nonlinear mobile phase electrical resistance. A characteristic parabolic profile is observed.
- This arrangement provides excellent characterization of gradient delay time
- The ability of providing the real-time mobile phase composition at the column inlet is useful, although high performance at compositions exceeding 50% ACN was difficult when using a current monitor with µA sensitivity. Nanoamp sensitivity would be preferable.
- Absolute measurement of flow rate by monitoring ground current with a step gradient should be possible using long (> 10 cm) grounding capillaries. This remains under investigation.

1. X. Huang, M. J. Gordon, R. N. Zare "Current-Monitoring Method for Measuring the Electroosmotic Flow Rate in Capillary Zone Electrophoresis" *Anal. Chem.* 1988, 60, 1837-1838.