

# Inline Electrospray 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 predominant 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 negatively 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 electrospray 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.

## Results

### Operating Principle:

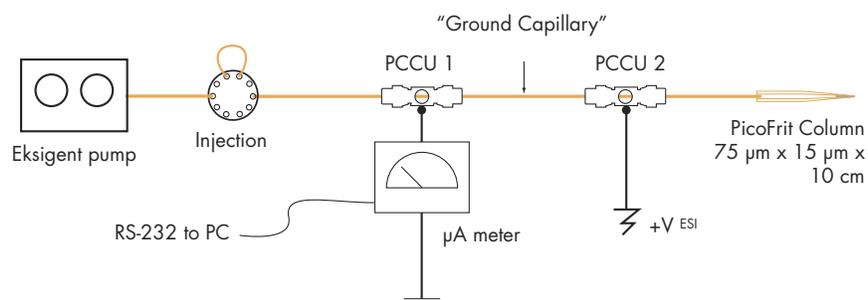
Electrical resistance of the mobile phase within a given section of capillary tube is a function of mobile phase composition. Due to efficient proton transfer, highly aqueous mobile phase (0.1% formic acid) exhibits relatively low electrical resistance. As the mobile phase becomes richer in acetonitrile, 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 electrospray 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)

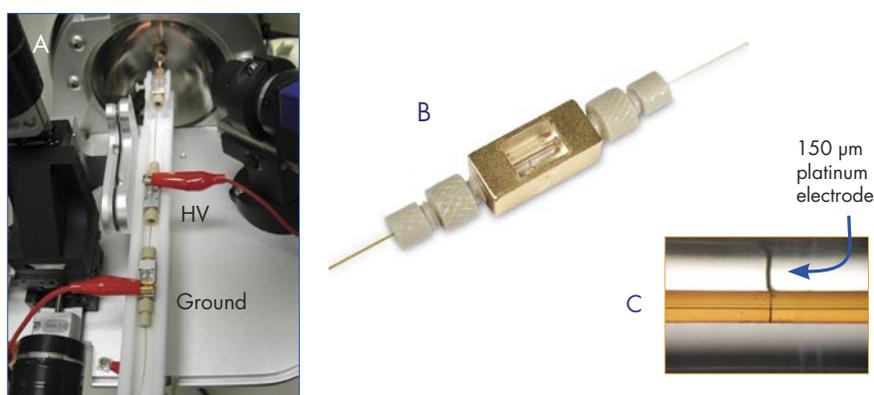
## Conclusions:

- 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.

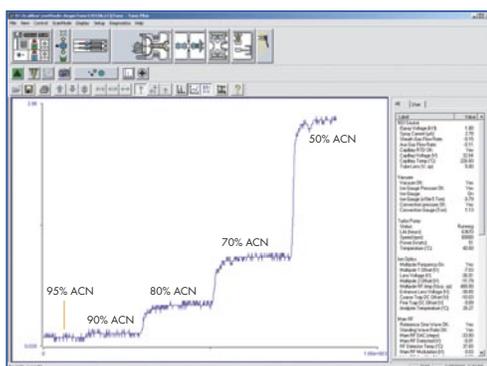
1. X. Huang, M. J. Gordon, R. N. Zare "Current-Monitoring Method for Measuring the Electroosmotic Flow Rate in Capillary Zone Electrophoresis" *Anal. Chem.* 1988, 60, 1837-1838



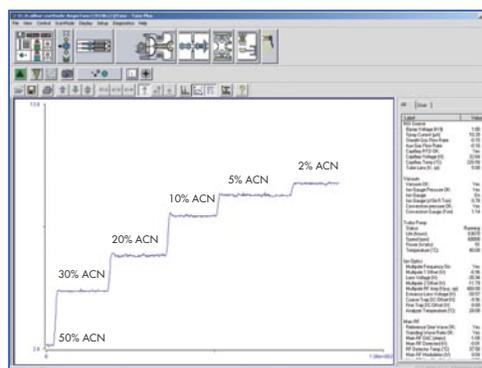
**Figure 1** Configuration schematic. The ground capillary carried a volume of either 100 nL (50  $\mu\text{m} \times 5\text{cm}$ ) or 220 nL (75  $\mu\text{m} \times 5\text{cm}$ ).



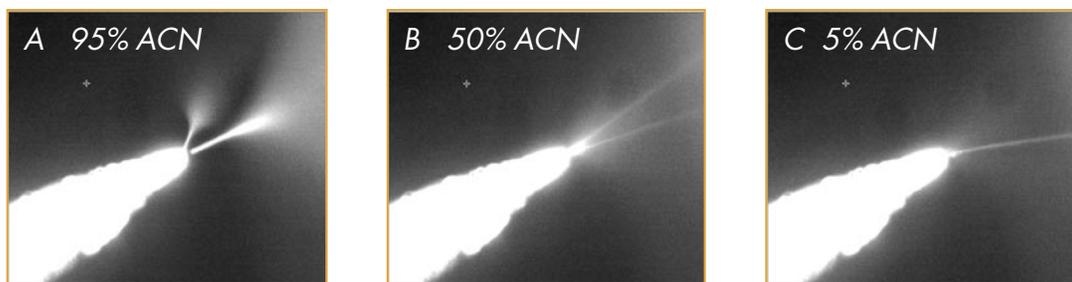
**Figure 2** A) Digital PicoView 150 nanospray source mounted on the Thermo Finnigan LCQ Deca mass spectrometer showing high-voltage and ground connections, B) PicoClear Conductive Union, and C) Voltage connection inside the PicoClear Conductive Union



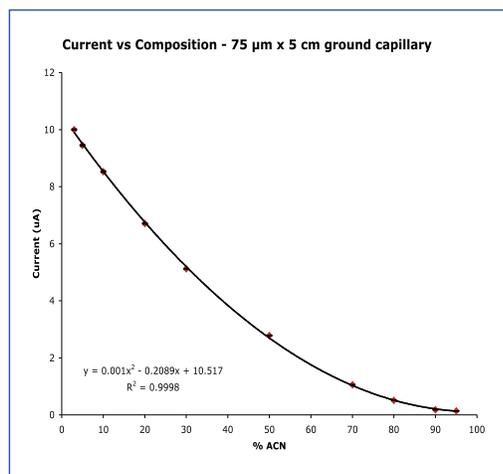
**Figure 3A** Calibration profile of current vs % ACN for a 75  $\mu\text{m} \times 5\text{cm}$  ground capillary. Nanospray source current was plotted against time. ESI voltage was 1.8 kV, flow rate of 500 nL/min.



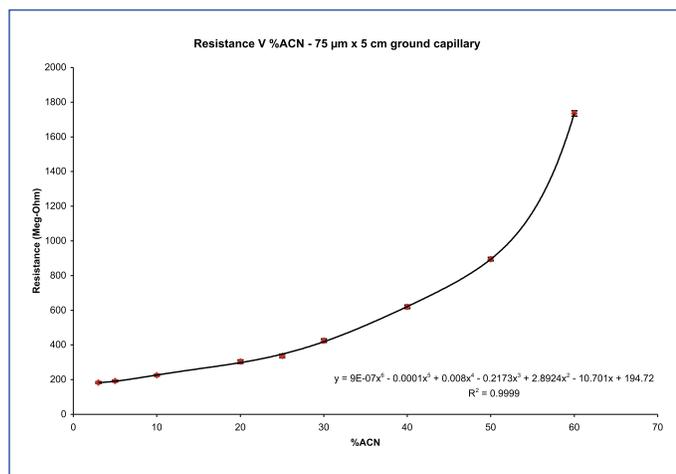
**Figure 3B** Calibration profile of current vs % ACN for a 75  $\mu\text{m} \times 5\text{cm}$  ground capillary. For compositions less than 50% ACN. ESI voltage was 1.8 kV.



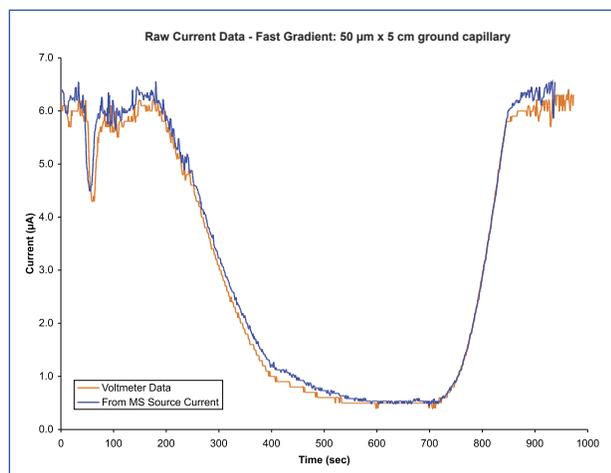
**Figure 4** ESI plume photos at A) 95%, B) 50%, and C) 5% ACN. Flow rate was 500 nL/min. through a 15  $\mu$ m PicoFrit emitter with an applied voltage of 1.8 kV.



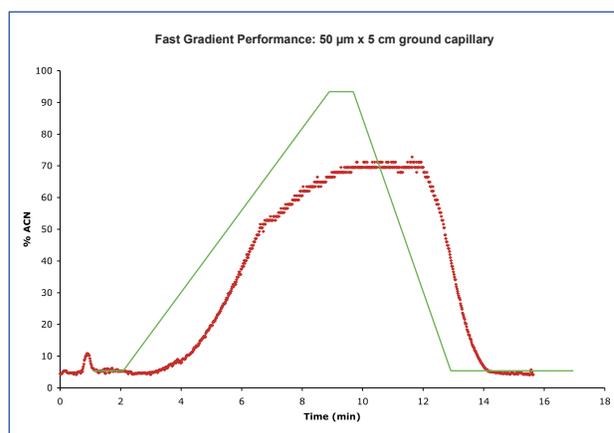
**Figure 5** Ground current vs % ACN for a 75  $\mu$ m x 5 cm ground capillary using the data from Figure 3



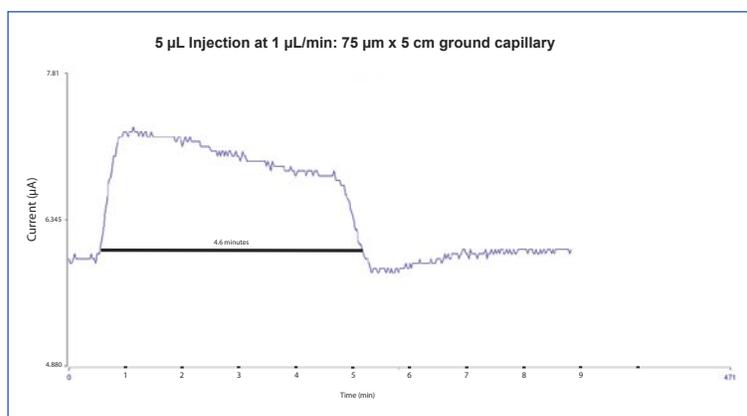
**Figure 6** The electrical resistance in M $\Omega$  vs % ACN for the 75  $\mu$ m x 5cm ground capillary. A high-impedance M $\Omega$  meter with a 2000 M $\Omega$  upper limit was used to measure the resistance. Mobile phase compositions > 60% ACN displayed resistance exceeding 2000 M $\Omega$ .



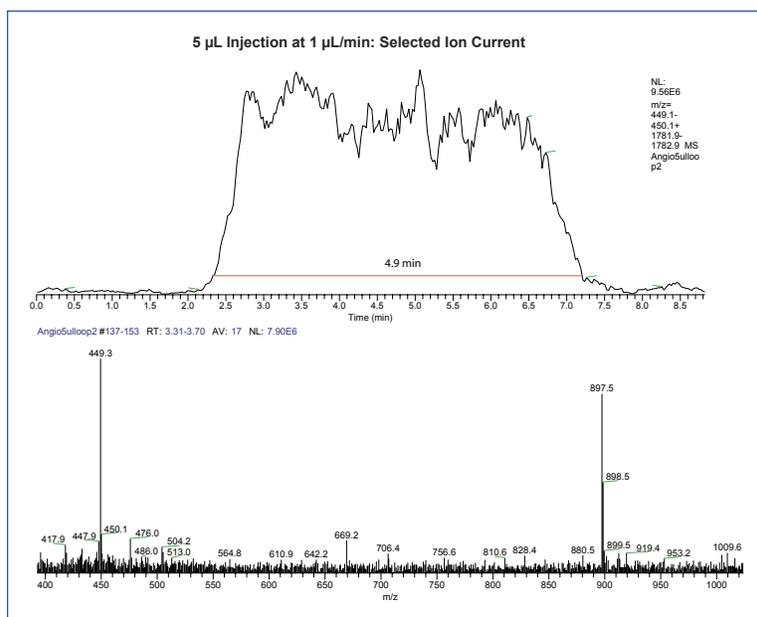
**Figure 9** Raw, unfiltered current traces obtained for a fast gradient at 500 nL/min. The electrospray voltage was 2 kV. The voltmeter data (orange) and the source power supply current (blue) show good agreement, indicating only a small portion of the source current is required to maintain the nanospray 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  $\mu$ A 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  $\mu\text{L}$  injection valve. A sample of two angiotensin peptides at 1  $\mu\text{M}$  concentration in 5% ACN was injected into a flow stream containing 30% ACN at a 1  $\mu\text{L}/\text{min}$ . 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.