

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

Adam W. Perala, Christopher J. Toher, Gary A. Valaskovic

New Objective, Inc., Woburn, MA

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 electrophoresis¹, 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.

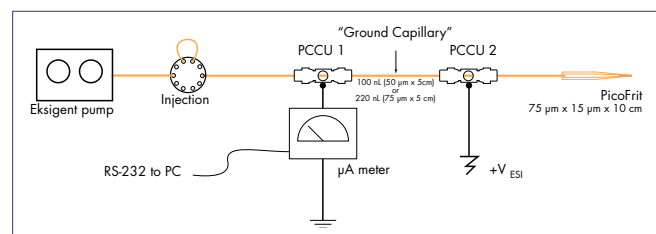


Figure 1 Configuration schematic

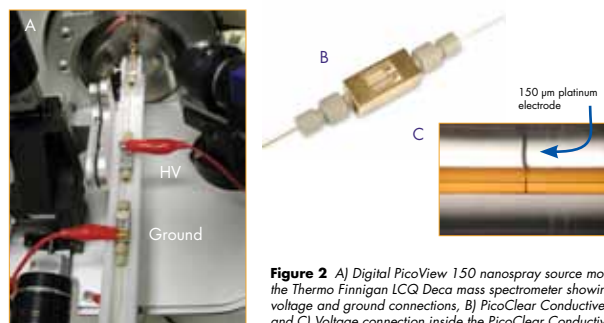


Figure 2 A) Digital PicoView 150 nanospray source mounted on the Thermo Finnigan LCQ Deca mass spectrometer showing high-voltage and ground connections, B) PicoClear Conductive Union, and C) Voltage connection inside the PicoClear Conductive Union

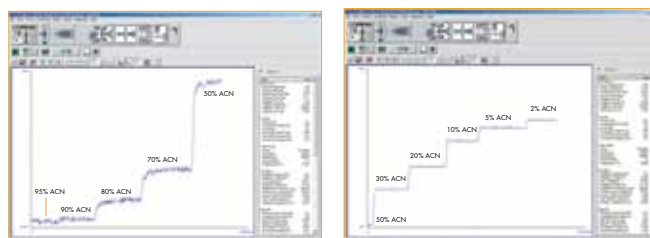


Figure 3A Calibration profile of current vs % ACN for a 75 µm x 5 cm ground capillary. Nanospray source current was plotted against time. ESI voltage was 1.8 kV, flow rate of 500 nL/min.

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.

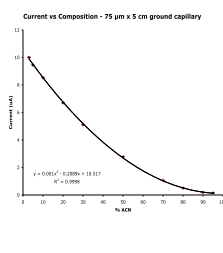


Figure 5 Ground current vs % ACN for a 75 µm x 5 cm ground capillary using the data from Figure 3

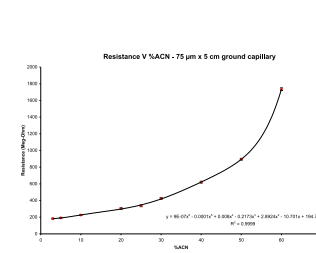


Figure 6 The electrical resistance in MΩ vs % ACN for the 75 µm x 5 cm ground capillary. A high-impedance MΩ meter with a 2000 MΩ upper limit was used to measure the resistance. Mobile phase compositions > 60% ACN displayed resistance exceeding 2000 MΩ.

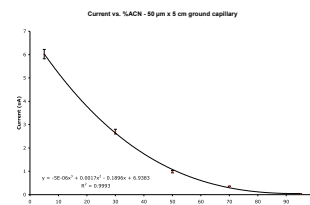


Figure 7 Ground current vs % ACN for a 50 µm x 5 cm ground capillary. The electropray voltage was 2 kV.

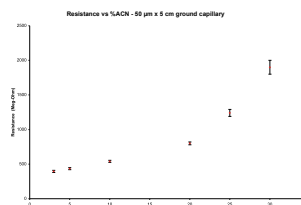


Figure 8 The electrical resistance in MΩ vs % ACN for the 50 µm x 5 cm ground capillary. A MΩ meter with a 2000 MΩ upper limit was used. Mobile phase compositions of greater than 20% ACN had a resistance of more than 2000 meg-ohm.

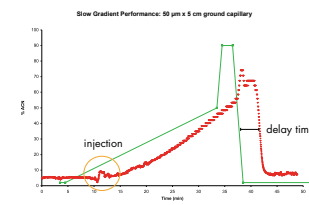


Figure 13 Calibrated %ACN vs. time obtained using the calibration curve from Figure 7. The programmed gradient is shown in green. Note: Because the µA meter is not sensitive above 70% ACN, the calibrated plot reaches a saturated value near 70%. The 0.5 µL injection plug is evident near the beginning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

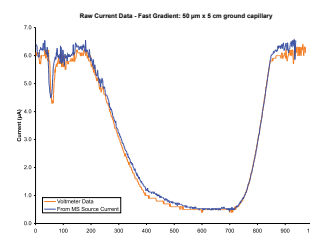


Figure 9 Raw, unfiltered current traces obtained for a fast gradient at 500 nL/min. The electropray voltage was 2 kV. The voltmeter data (orange) and the source power supply current (blue) show good agreement, indicating only a small portion of the source current is required to maintain the nanospray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

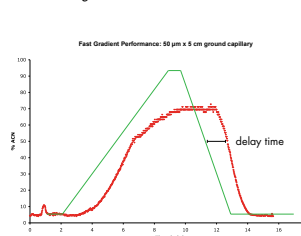


Figure 10 Application of the calibration curve (Figure 7) to the current trace (Figure 9) yields a plot of % ACN vs time. The programmed gradient is shown in green. Note because the µA meter is not sensitive above 70% ACN, the calibrated plot reaches a saturated value near 70%.

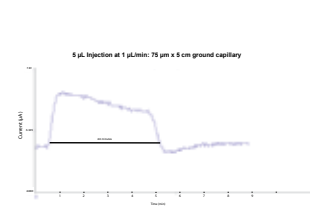


Figure 11A A current trace for an injection plug from a 5 µL injection valve. A sample of two angiotensin peptides at 1 µM concentration in 5% ACN was injected into a flow stream containing 30% ACN at a 1 µL/min. flow rate.

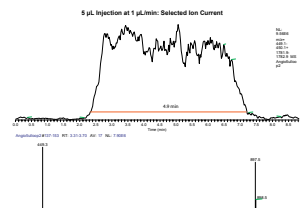


Figure 11B Mass spectral acquisition of the injection profile from Figure 11A. Note the current monitor provides a similar time profile, suggesting the current monitor may be useful in monitoring on-column sample injection.

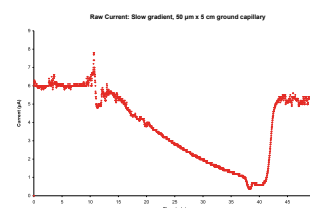


Figure 12 Raw, unfiltered current trace obtained for a traditional slow gradient at 300 nL/min from a 50 µm x 5 cm ground capillary. The electropray voltage was 2 kV. The gradient-programmed profile is shown in Figure 13.

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.