NanoLC Fraction Analysis by Chip-Based Nanoelectrospray

CNF Project # 740-98
Principal Investigator: Dr. Thomas N. Corso

Abstract:
In previous reports we have shown our progress in the development and commercialization of a monolithic microfabricated microfluidic device for the purpose of conducting nano-electrospray ionization (nESI) when coupled to a mass spectrometer. We have recently developed further fluidic handling capabilities for users of nano liquid chromatography columns (nanoLC) and created the first ultra-low volume fraction collection technique for nanoLC with subsequent nanospray infusion analysis for extend MS analysis time targeted at glycopeptide analysis.

Summary:
NanoLC with 75 µm id columns and flow rates of 200 nL/min is gaining in popularity due to improved resolution, lower sample injection requirements, and better ionization efficiency leading to improved sensitivity. NanoLC peaks typically elute within 20 sec, providing most modern mass spectrometers sufficient time to perform MS/MS for simple protein ID experiments. However, for complex samples, such as glycopeptides, where MS3 or MS4 experiments may be needed, nanoLC does not provide adequate analysis time.

By collecting fractions from the nanoLC, analysis times can be extended via nanospray infusion analysis. Fractions from a nanoLC were collected into pipette tips, these 200 nL fractions were collected every 60 sec from a column flowing at 200 nL/min with a 30 min gradient. Fractions were collected from the peak elution window of interest in an automated fashion using the NanoMate. The nanoLC fractions in the pipette tips dried within several minutes. Following fraction collection the residue in each tip was reconstituted. The sample was analyzed directly from the tip with chip-based nanoelectrospray using our ultra low flow chip. This chip has 3 µM id nozzles, producing flow rates of < 40 nL/min and providing 8 min analysis per fraction. Fraction collection, reconstitution and analysis steps were fully automated.

To demonstrate the system, Ribonuclease B (RNaseB) tryptic digest, which is known to have a single N-linked glycosylation site on the asparagine 34 residue, was used. 5 pmol of RNaseB tryptic digest was injected onto a 75 µm x 360 µm x 15 cm operated at a flow rate of 200 nL/min. 60 sec nanoLC fractions (200 nL) were collected into pipette tips by robotic capillary insertion. During the collection time a solvent droplet on the end of the capillary grew, and at the end of the collection time, the capillary was withdrawn from the pipette tip, capturing the droplet inside the end of the pipette tip. For the RNaseB analysis 24 fractions were collected. The 200 nL fractions in the tips dried in minutes and a reconstitution step was performed with a desired solvent composition. 75% methanol in water with 0.1% acetic acid was added to wells of a 96-well plate.

The NanoMate robotically aspirated 500 nL into a pipette tip containing a fraction, and then waited 75 sec before engaging the tip to the chip and performing the infusion analysis. The purpose of this delay period was to allow for both solvation of the LC fraction and evaporation of the reconstitution solvent to reduce volume and concentrate the sample. It was estimated that the final volume of solvent was approximately 300 nL and the composition was 50% methanol. The analyses were performed on an LCQ Deca XP ion trap equipped with a NanoMate. MS3 and MS4 experiments were performed on the nanoLC fractions containing isoforms of the glycopeptide. The extended analysis time of each fraction allowed for collision energy optimization, data averaging, and multiple tandem MS experiments. The site of glycosylation was determined, the PTM was characterized as having 9 mannose and 2 N-acetylglucosamine groups, and the sequence of the peptide in which the glycosylation occurred was determined. This approach will be demonstrated as a useful means of extending analysis times for nanoLC.
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Principal Investigator: Dr. Thomas N. Corso
Users: Christopher G. Alpha, Thomas N. Corso, Jie Li, Barry L. Smith

Affiliation: Advion BioSystems, Inc.
Primary Funding: Advion BioSystems, Inc.
Contact: calpha@advion.com, tcorso@advion.com, jli@advion.com, bsmith@advion.com
Web Site: http://www.advion.com/

Figure 1, opposite left: (A) NanoLC fractions are collected into pipette tips when the fused silica column tail is inserted through the end of a pipette tip by the NanoMate. A droplet of column effluent grows until the pre-determined collection time has been reached. (B) Then the fused silica is quickly retracted and the effluent is captured at the tip of the pipette.

Figure 2, below left: (A) NanoLC chromatogram of 100 fmol of RNase B tryptic digest. Four 90-second nanoLC fractions were collected in the ranges indicated. Glycopeptides were found in tips #2 and #3, but there was no evidence of them in tips #1 or #4, showing that the chromatography is preserved in the nanoLC fractions. (B) The full scan MS spectrum from tip #2 (shown in Figure 9A) shows the presence of five glycopeptides in the nanoLC fraction, varying from five to nine mannose groups.

Figure 3, above: Cross-section cartoon depicting of the fluid interface for the nanoelectrospray chip. A micropipette tip supplies the sample to the back of the chip’s inlet. Inset: SEM image of the 2.5 µm id nanoelectrospray nozzle.