

High Sensitivity Nanospray Chip Nozzles for Direct Infusion and Online NanoLC

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Overview

Nanospray mass spectrometry provides high sensitivity results because the unassisted spray creates lower background and possibly higher ionization efficiency. Conventional nanospray sources such as highly tapered capillaries and microfabricated silicon devices restrict the samples to those that are in fairly clean matrices to prevent clogging, and the flow rates to those typically under 1 microliter/minute in order to take advantage of the high sensitivity of nanospray. For on-line nanoLC-nanospray applications, the clogging of the nanospray source is a major stumbling block to successful experiments.

A plastic nanospray chip nozzle has been designed to be fundamentally different from these conventional sources. Each nozzle has a 50-micron o.d. and a 20-micron i.d. opening, and the nozzle is connected to a short conical channel that opens to 360 microns within 1 mm. The relatively large electrically insulating nozzle and the non-restrictive flow channel allow samples in complex matrices such as diluted but unfiltered sera and fermentation broths to be sprayed successfully, and online nanoLC-nanospray-MS to be robustly carried out over the entire range of mobile phase compositions with no fittings between the spray nozzle and the column. The same system may also be used for loop-injection-nanospray system (with the nanoLC column replaced by just an open tube) to take advantage of the large flow rate range afforded by the nanospray nozzle.

In this reports, the performance of the nozzle was evaluated during nanoLC experiments of protein and cell digests. The results are presented alongside those obtained with the conventional commercially available Pico frit (from New Objective, Woburn, MA) nanospray sources for comparison.

Method

Columns: 360 µm outside diameter (o.d.) fused silica capillaries with 50 µm, 75 µm or 100 µm i.d.'s were used. The frit was made according to the sol-gel Kasil protocol. Commercial Pico frit capillaries were used for comparison. Columns were packed with 3 µm C18 particles to a depth of 10 cm, or 1.8 µm C18 particles to a depth of 15 cm. The column was cleaved off with a silica column cutter for a clean, square-cut end to be inserted leak-free into the nanospray nozzle.



A nanoLC packed column inserted into a sliced nozzle

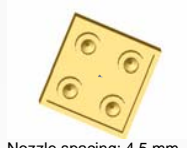


A nanoLC packed column inserted into a punched-out nozzle

Nanospray nozzle and chip design



- Conical nozzle structures 0.5 mm to 3.5 mm in height
- 20+/- 3 µm i.d., 50 µm o.d.



Nozzle spacing: 4.5 mm

- A reservoir of a few microliters connects directly to each nozzle through a conical channel the entrance of which can form a liquid-tight junction with a 360 µm o.d. column.
- Four nozzles per chip, 4.5 mm spacing

- Chips can be tiled to any configuration up to a 384 microtiter-plate

- Each chip can be either sliced or punched out into 4 individual nozzles for applications in nanoLC or direct infusion.

NanoLC Experimental

Nanospray-Mass spectrometry interface:

All the experiments here were performed with the individual nanospray nozzle. The column inserted into the back of the nozzle held the nozzle in position for optimized spraying. It was not necessary to have an electrical ground to the nozzle.

Electrical contact between the sample and the high spray voltage was made through a liquid T-junction before the column. A voltage of 1.3 KV was applied through a gold wire to the sample. The nozzle was placed 0.5 to 2 mm from the mass spectrometer inlet.

The mass spectrometer used was a ThermoFinnegan LTQ



The nanoLC column with integrated nozzle was held with a holder which was in turn placed on the nanospray interface of the MS. HV was supplied through a T junction.



The nozzle was placed about 1mm from the MS inlet so that the entire effluence of the column was sprayed into the MS inlet to maximize sensitivity.

LC conditions for the 3 µm particle columns

50-65 microliters/minute from HPLC was split to obtain the desired flow rates, 250 nL/min and 500 nL/min through the column in the data shown here.

Mobile Phases for LC:

- A: H₂O + 0.1% Formic Acid
- B: Methanol + 0.1% Formic Acid.

A typical run for a column with C18 particles was as follows:

- 1)10%B for 10 minutes (sample loading and column washing), Single nozzle supported by column
 - 2)10%-85%B gradient for 50 minutes
 - 3) 90% B for 10 minutes
 - 4) 2 minute gradient back to 10%B
- Data were collected over the entire 72-minute run.

LC conditions for the columns with 1.8 µm particles

A splitless nanoflow HPLC was used for the 1.8 µm particle columns. 75 µm x 15 cm columns were used here at a flow rate of 500 nL/min.

Mobile Phases for LC:

- A: H₂O + 0.1% Formic Acid
- B: Methanol + 0.1% Formic Acid.

A typical run for a column with C18 particles was as follows:

- 1)10%B for 10 minutes (sample loading and column washing), Single nozzle supported by column
 - 2)10%-20%B gradient for 5 minutes
 - 3) 20-50% B gradient for 60 minutes
 - 4) 50-85%B gradient for 15 minutes back to 10%B
 - 5) 10 minutes at 85% B
- Data were collected over the entire 100 minute run.

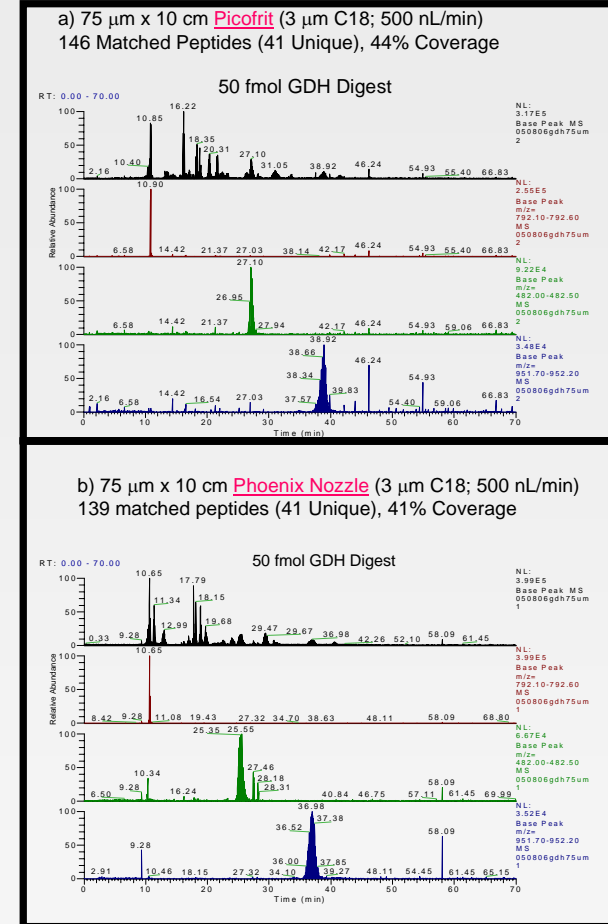
NanoLC samples

For the column with 3 µm particles: 50 fmol of trypsin-digested glutamate dehydrogenase (GDH)

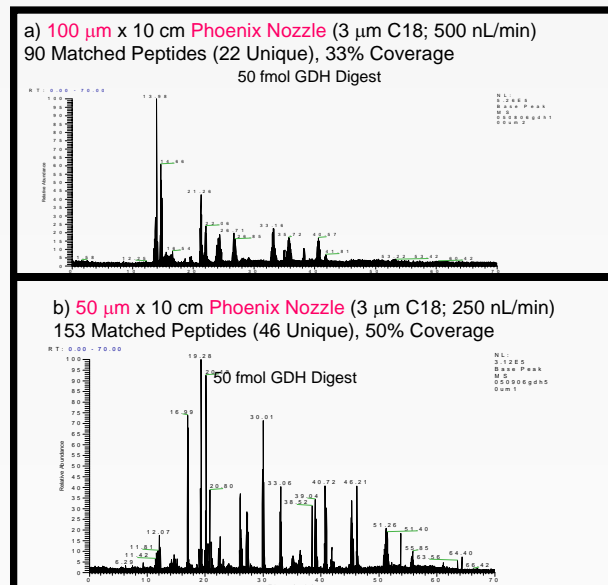
For the column with 1.8 µm particles: HeLa cell lysate digest

Results

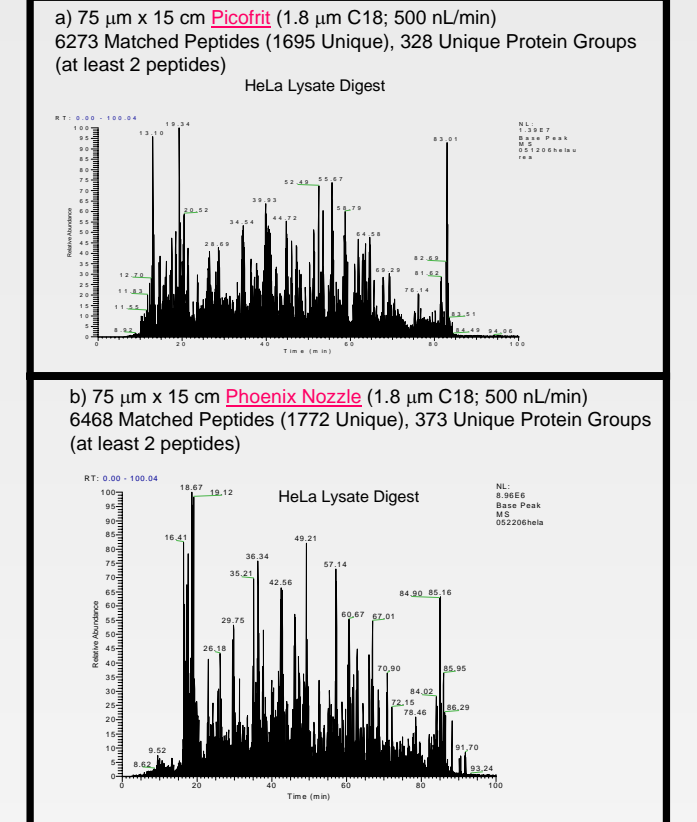
1) The following chromatograms compare the sensitivity and resolution of the LC results between the Pico frit and the nanospray nozzle. The peak widths and sensitivity of both sources can be seen to be comparable.



2) The second set of results was obtained with the nanospray nozzle attached to columns of different inside diameters, i.e., 50 µm vs. 100 µm.



3) The third set of results was obtained with columns packed with 1.8 µm particles and sprayed with the Pico frit source vs. the nanospray nozzle.



Summary and Discussions

The results reported here clearly show that the performance of the nanospray nozzles at least matched if not exceeded conventional nanospray sources. The added benefits the nanospray nozzle provides are:

- Analytical flexibility was shown in the second set of results where columns of a variety of inside diameters were used with the nozzle. In this instance, the 50 µm i.d. column is shown to have clear advantages over the 100 µm column.

- Although not directly derivable from the results shown, the nozzle is very robust and each one can last several months or more because of its clog resistance property. In fact, all the data shown here used a single nozzle.

- The sensitivity and resolution of the column equipped with the nanospray nozzle can be further improved by tapering the end of the column to take up the ~ 29 nL of dead volume in the conical channel leading to the nozzle opening. Preliminary results obtained with the tapered column (not shown in this report) show that the full peak width may be reduced by over 60%.



A column where the end with frit was tapered so that the taper could be inserted deep into the conical channel of the nanospray nozzle to reduce the dead volume to 2-5 nL

Acknowledgements: Phoenix S&T, Inc. wishes to thank Noel Whitaker of the U. of Maryland at College Park for assistance with column preparation and helpful discussions.