Large Flow Rate Range of Polypropylene-based Nanospray Nozzle Chips

S. L. T. Staats, J. W. Ashmead, A. Suna, Phoenix S&T, Inc.

A. J. Fogiel and A. T. Gutsche, DuPont Central Research and Development

Overview

Nano-electrospray (nanospray) provides high ionization efficiency and consumes nanoliters of samples. Conventional nanospray sources made of pulled capillaries for mass spectrometry are typically capable of delivering a small range of flow rates for a given source opening, e.g., 20- 100nL/minute, 100-500 nL/min., etc. The flow rates are generally believed to be directly correlated with the inside diameter of the tip opening. Low flow rate (under 50 nL/min) nanospray, in addition to the obvious advantage of conserving scarce samples, offers desirable performance advantages such as higher sensitivity and higher tolerance for salts in the samples. However, since low flow-rate sources typically have tip openings of only a few microns, extreme care and effort must be taken to rid samples of particulates and contaminants to minimize clogging.

We investigated the flow rate range applicable to the new polypropylene nanospray chip, which has a geometry dramatically different from the pulled capillary sources. The same 20-micron i.d. nozzle was used to spray at flow rates from <25 nL/min to very high rates of ~ 5 μm/min. Sensitivity vs. flow rates from 2 μL/min. to 12.5 nL/min. was carried out using standards with a single nozzle. The plumes of the sprays at varies flow rates were imaged. The ability of the plastic nozzle to provide a stable spray at low flow rates (<50 nL/min.) without clogging the nozzle was exploited in a metabolic screening experiment where a "dilute-and-shoot" method was developed to detect a metabolite in a 20% culture broth (LB broth) solution without previous sample clean-up steps. Our observations will be discussed in terms of cone-jet spray mode and the Taylor cone formation in the unique geometry of the plastic nozzle design.

Methods

Nanospray Nozzle and Chip Design

- Conical nozzle structures 0.5 mm to 1.5 mm in height
- 20+/- 3 μm i.d., 50 μm o.d.
- A reservoir of a few microliters to milliliters connects directly to each nozzle
- Capillary from a syringe pump plugs directly to the nozzle without fittings
- Four nozzles per chip
- 384 microtiter-plate format

Experimental setup

For the flow rates vs. sensitivity studies, the following two systems with two different kinds of mass spectrometers were used.

System 1: Gramicidin S, 2 μg/mL concentration in 50/50 methanol water.

Mass spectrometer: Micromass Ultima triple quadrupole in full scan mode;

Positive ion spray using a syringe pump (Harvard model 11) and 5 or 10 μL capacity syringes

System 2: 4-component standard: caffeine (MW=177.13), Desipramine hydrochloride (MW=266.19), peptide 1 (MW=494.16), peptide 2 (MW= 863.44) in 60/40 ACN/water.

Mass spectrometer: Mariner TOF in full scan mode; positive ion spray using the built-in syringe pump and a 10 μL capacity syringe.

For the broth study, the standard used was 6 μM parahydroxycinnamic acid (PHCA, MW=164) in a buffer containing 10 mM ammonium acetate, and 50/50 water/isopropanol (IPA). The culture broth was LB broth.

Mass spectrometer:Micromass Ultima triple quadrupole in MRM mode monitoring daughter ion m/z= 129;

The negative ion spray was carried out with a syringe pump (Harvard model 11). The flow-rate was 50 nL/min., The spray was orthogonal to the mass spectrometer inlet.

The flow rates were verified by calibrating the emptying rates of the syringe with time. The syringe pump used (Harvard) generated significant mechanical noise for flow rates below 40 nL/min for a 10 μL capacity syringe, or 25 nL/min for a 5 μL capacity syringe.

Results

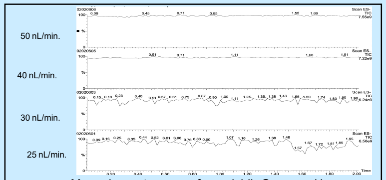
Spray Stability and Sensitivity vs. Flow Rates

50 nL/min.

40 nL/min.

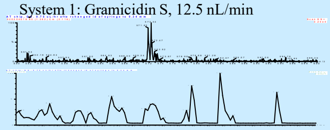
30 nL/min.

25 nL/min.



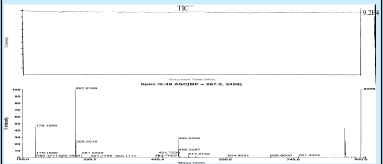
Mass chromatograms of gramicidin S pumped by a syringe pump on a 10 μL capacity syringe. The flow rates were from the bottom: 25 nL/min., 30 nL/min/, 40 nL/min. and 50 nL/min. The noise in the traces decreased as the flow rate increased. The noise in the 25 nL/min. trace can be correlated with the turning of the screw of the syringe pump.

System 1: Gramicidin S, 12.5 nL/min



Top trace: Mass spectrum showing the m/z=571 cluster of peaks characteristic of gramicidin S. Bottom trace: Mass chromatogram of a 2 minute, 100- 1000 amu full scan run. At this low flow rate, the rod of the syringe pump was turning discontinuously thereby generating a discontinuous spray from the nozzle. The 5 μL capacity syringe was used in this experiment. The signal of the m/z= 571 peak was still significantly above noise at this low flow rate.

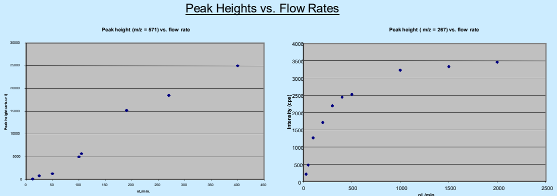
System 2: 4-component standard, 2 μL/min



Top trace: The total ion current for the 4-component standard. Total scan time was 1 minute.

Bottom trace: The mass spectrum of the 4-component standard. The peak height of m/z = 267 was used to represent sensitivity in the plot of peak height vs. flow rates.

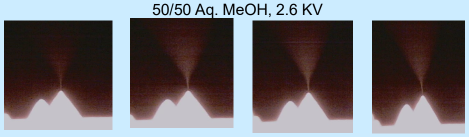
Peak Heights vs. Flow Rates



Spray Characteristics vs. Flow Rates

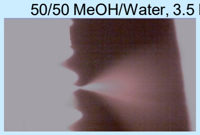
- Cone-jet mode was observed over the entire range of flow rates investigated, and of all materials sprayed: pure aqueous, 50/50 water/ACN or methanol, high salt content diluted broth, etc.

50/50 Aq. MeOH, 2.6 KV



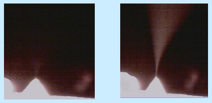
25nL/min 500 nL/min 1000 nL/min 1200 nL/min

50/50 MeOH/Water, 3.5 KV



5 μL/min

Distilled water, 3 KV

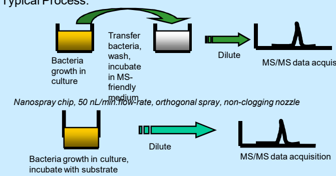


50 nL/min. 200 nL/min.

Application

Bioprocess Monitoring: MS of metabolites in broth –"dilute-and-shoot" with the plastic nanospray chip

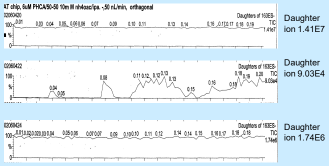
Typical Process:



MS chromatograms of broth+metabolite

50 nL/min. flow rate, orthogonal spray, -1.8 KV, MS-MS

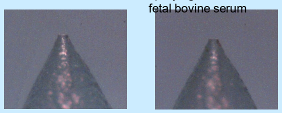
Good signal to nose intensity of the metabolite daughter ion in the diluted buffer



Non-clogging Plastic Nanospray Nozzles

Before use

No deposits observed after spraying unfiltered, diluted (100x) fetal bovine serum



Discussions

Flow Rates vs. Nanospray Chip Design

We have tried to relate flow rates and nozzle dimensions with the aid of cone-jet theory as found in the aerosol literature. In that theory, the flow rate determines the size of the jet and the size of the transition region, where the shape of the meniscus deviates significantly from a Taylor cone. It is reasonable to suppose that the flow rate Q must not exceed the rate at which the radius rt of the transition region[1]:

$$rt = \left(\frac{Q\,\beta\,\epsilon 0}{K}\right)^{1/3}$$

becomes comparable to the nozzle radius. Here ε_0 is the permittivity of free space (8.85 x 10^{12} farad/m), β is the relative dielectric constant, and K is the conductivity in S/m, and Q is expressed in m^3/sec . We have found that, for the values of these quantities pertinent to our test solutions, rt is much smaller than our smallest nozzle openings at all observed flows. Thus our maximum flows are not limited by our nozzle dimensions.

$$Qmin = \frac{\sqrt{-1+\beta}\;\gamma\,\epsilon 0}{K\,\rho}$$

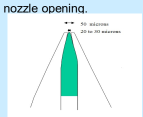
cone-jet theory predicts that a stable jet can be formed only when the flow exceeds the value Qmin given by [2]:

where γ is the surface tension (N/m) and ρ is the density (Kg/ m^3). This does not depend on any geometric constraints, such as nozzle dimensions. For our test solutions, Qmin is always well below the lowest flow rates.

If we apply these cone-jet formulas to the conventional glass capillaries, we find similar results, so there ought not be the observed restrictions on flow rates vs. nozzle openings. We can only speculate why such restrictions seem to exist. Perhaps larger flow rates in small nozzles are inhibited by the inherently more constricted supply capillaries, while other practical concerns seem to preclude very low flow rates for larger capillaries.

There is another feature of our polypropylene nozzles which makes them superior to glass nozzles. We have estimated that, because polypropylene is hydrophobic, it is possible to form a Taylor cone inside the nozzle, as capillary action can exceed the opposing electric force. For hydrophilic glass, capillary action will instead aid electric forces in pulling the meniscus to the opening. The result of this is that only in our nozzles, a high speed jet can be formed inside the nozzle before the nozzle has narrowed to the point of potential clogging at low flows. Microscopic observation of our sprays has confirmed such interior cone formation. In addition, we have seen insensitivity of the onset voltage to the nozzle opening [3]. The latter can then be explained by our earlier contention 3that the radius which determines the onset voltage is that of the meniscus of the electrically charged liquid, not necessarily the radius of the nozzle opening.

A schematic drawing showing the geometry inside the insulating plastic nozzle filled with an electrically charged liquid (in green). The Taylor cone may be formed inside the nozzle opening.



Conclusions

- Plastic nanospray chip is capable of cone-jet mode nanospray over a large range of flow rates, from 25 nL/min. to 5 μL/min.
- The nozzles are clog-resistant even at very low flow rates (<50nL/min and are therefore suitable for analytes in a "dirty" medium. No deposits were observed on the nozzles after prolonged (>10 minutes) spray of unfiltered broth and serum, making 'dilute and shoot" method development possible.
- Sensitivity as represented by peak height increases with flow rates from 25 nL/min. to about 1000 nL/min. No enhanced sensitivity was observed at flow rates lower than 50 nL/min.
- The large flow rate range of the plastic nozzle is consistent with cone-jet sprays.

References:

1. L.T. Cherney, J. Fluid Mech. Vol.378, pp 167-196, 1999
2. A.M. Gañan-Calvo, J. Dávila, and A. Barrero, J.Aerosol Sci., Vol.28, No.2, pp 249-275, 1997.
3. S. L. Tang Staats, D. Chow and A. Suna, Proceedings of the ASMS Conference 2003

Acknowledgements: Phoenix S&T, Inc. wishes to thank the DuPont Company for the use of DuPont laboratories in support of this poster however acknowledges that this support in no way should be construed as an endorsement by DuPont of Phoenix S&T, its products or services. Phoenix S&T, Inc. also wishes to thank Dr. Tom Solsten and Dr. Harald Schroeder of Aventis Pharmaceutical for their help to collect and permission to present some of the data.