Large flow rate range of polypropylene-based nanospray nozzle chips

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Nano-electrospray (nanospray) provides high ionization efficiency and consumes mere 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 polypropylene nanospray nozzles are conical structures 0.5 mm to 1.5 mm in height. The inside diameter of the nozzle is 20+/- 3 µm, and the outside diameter of 50 µm. A reservoir of a few microliters connects directly to each nozzle. For the present experiments, a capillary from a syringe pump plugs directly to the nozzle without fittings to accurately pump sample through the nozzle at various flow rates. For the flow rates vs. sensitivity studies, two different systems were used. System 1 used Gramicidin S, (2 µg/mL concentration) in 50/50 methanol water as the standard. The mass spectrometer used was a Micromass Ultima triple quadrupole in full scan mode. Positive ion spray was carried out using a syringe pump (Harvard model 11) and 5 or 10-µL capacity syringes. System 2 used a 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. The mass spectrometer used was a Mariner TOF in full scan mode. Positive ion spray was carried out 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 50/50 10 mM ammonium acetate (adjusted to pH 8.5) /isopropanol (IPA). The culture broth was LB broth. The Mass spectrometer was a Micromass Ultima triple quadrupole in MRM mode monitoring daughter ion *m/z*= 119. The negative ion spray was carried out with a syringe pump (Harvard model 11) and a 10 µm capacity syringe. 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 pumps used 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.

The results showed that the same 20-micron i.d. nozzle could be used to spray at flow rates from <25 nL/min to very high rates of ~ 5 µL/min. Sensitivity vs. flow rates from 2 µL/min. to 12.5 nL/min. was carried out using standards with a single nozzle. The peak height of the dominant m/z peak was used to represent sensitivity, which was found to increase with increasing flow rates up to about 1 µL/min., at which point the peak height remained more or less constant as the flow rate was increased further. The plumes of the sprays at various flow rates were imaged and could be easily determined to be the cone-jet mode. 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 simulated metabolic screening experiment where a "dilute-and-shoot" method was developed to detect an analyte in a 20% culture broth (LB broth) solution with only a 1:1 dilution step in IPA before MS analysis. Figure 1 below shows the MS/MS results of the daughter ion of the analyte standard in the diluted (1:10) LB broth (bottom panel). The intensity of the daughter ion in the diluted broth was substantially lower than that (10^6 cps) in the buffer alone (10^7 cps), indicating that ion-suppression took place in the complex broth mixture. However, the signal to noise (the background (10^5 cps) was shown in the middle panel) ratio of the daughter ion was still excellent making it possible to monitor the metabolite in the bioprocess without the extra step of transferring the bacteria out of the broth environment into an artificial MS-friendly medium for the eventual MS sampling.

Our observations are discussed in terms of cone-jet spray mode and the Taylor cone formation in the unique geometry of the plastic nozzle design. We 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. For minimum flows, cone-jet theory predicts that a stable jet can be formed only when the flow exceeds the value Qmin given by [2]

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

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 used experimentally.

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 that 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.

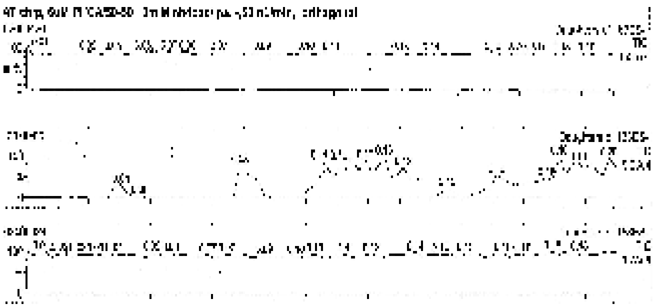

Figure 1 : Mass chromatogram of the analyte in 50:50 diluted (20%) broth in buffer:IPA. The flow rate was 50 nL/min. The spray voltage was –1.8 KV. The spray was orthogonal to the mass spectrometer inlet to minimize contamination by the broth. The top panel is the daughter ion (m/z=119) intensity in the 50:50 buffer:IPA. The middle panel was the background signal of the m/z = 119 in the diluted broth and IPA. The bottom panel was the signal of the daughter ion in the diluted broth and IPA.

References:
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