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A NOVEL NANOSPRAY EMITTER DESIGN WITH A CUSTOM INTERFACE FOR NANO-LC/MS

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OVERVIEW

- A novel nanoelectrospray emitter has been designed to address the shortcomings of fused silica pulled tip emitters.
- The newly designed emitter is durable, robust, adaptable for a wide range of flow rates and can be adopted to any MS platform.
- The emitter can quickly be regenerated if an unstable spray is observed or if the emitter clogs.
- A nanoflow sprayer was designed to incorporate the new emitters to a commercially available MS platform.
- The emitter/sprayer combination provides a stable and sensitive spray for nLC-MS analysis.

INTRODUCTION

A widely used method for protein/peptide identification incorporates nanobore liquid chromatography (nLC) with nanoelectrospray ionization mass spectrometry. This combination has proven to provide superior detection limits compared to conventional LC due to low elution volumes. The most common nanoelectrospray apparatus used by mass spectrometrists employs a sharp needle or emitter fashioned from fused silica tubing with a conductive outer layer. Coupling a nanospray emitter with a nanobore column is critical to an analysis as insufficient column emitter coupling can lead to band broadening and loss in sensitivity. Packed pulled fused silica emitters have overcome this problem but the integral format has a limited lifetime due to the fragility, loss of a conductive coating on the emitter and frequent clogging of the tip. Here we present a novel emitter design that addresses these issues.

The NanoEase emitters* provide the following features and advantages:

- Regeneration capability
- Physically robust
- Pre-cut, diamond- polished distal end
- Adaptable to wide range of column diameters and flow rates
- Can be incorporated on any nanospray platform

A Universal NanoFlow™ sprayer* was designed specifically to work with the new emitters. The sprayer has the following key features:

- Zero dead volume metal union which minimizes both post-column band spreading and loss in sensitivity
- Adjustable slider for accommodating different emitter lengths and for spray optimization
- Sheath gas capability
- User ease of use (finger tight nano-fittings)
- Minimal time required for tip replacement and regeneration

* Patent Pending
NanoEase Emitter P/N: 186003298
Universal NanoFlow Sprayer P/N: 289001685

The nanospray emitter is composed of a fused silica body with 360 µm OD and an emitter with 90 µm OD and 20 µm ID. Conventional nanobore fittings and unions may be utilized to connect the emitter body to an adjacent piece of fused silica or PEEK without the use of special sleeves. The emitter is flexible and robust which allows for easy handling and storage (Figure 1).

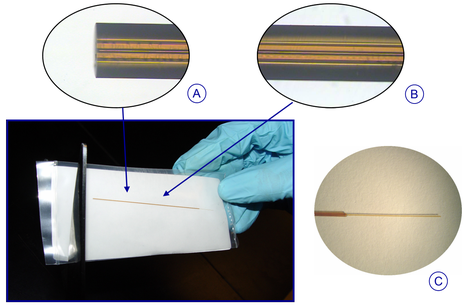

Figure 1. Nanospray emitter - Removal of a NanoEase emitter from a sealed bag. Microscopic images are shown of the emitter's diamond polished distal end (A), body (B) and tip (C).

A nanospray probe was designed to incorporate the new emitters to a commercially available MS platform. The compact sprayer head profile allows fused silica emitters of 5 to 7 cm lengths to be used in conjunction with an optional sheath gas (A in Figure 2). A counterbored nut and a ferrule holds the emitter and creates a seal which directs a sheath gas over the tip of the emitter. The sprayer features a zero-dead volume union which is held in place with an adjustable stop (B in Figure 2). The ZDV union allows a direct end to end connection between the emitter and a 360 µm column or transfer line, minimizing post column band spreading. Properly centered connections are made with the aid of a gauge plug (Figure 2, right). The union may also be easily removed for cleaning or replacement.

A spray can be generated from an emitter by placing an elevated potential anywhere on the sprayer as the metal union serves as a liquid junction contact for uncoated nanospray emitters.

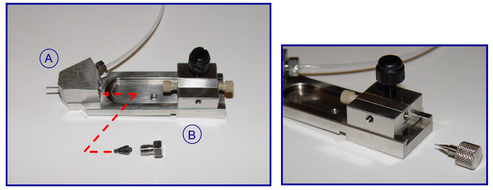

Figure 2. Nanoflow Sprayer

METHODS

- Mass Spectrometer:
  A Q-Tof micro™ was utilized to test the long term stability of the NanoEase emitter implementing direct infusion
  A Q-Tof Premier™ (V mode, R=11,500) was utilized to display the regenerative feature of the emitter and study its performance with gradient chromatography
- LC:
  nanoACQUITY™ UPLC
  Solvents: A: 0.1% formic acid, B: acetonitrile w/ 0.1% formic acid
  Gradient: 2%-60% B in 30 min
- Nanospray source:
  NanoLockSpray source with a nanoflow sprayer (Figure 3)

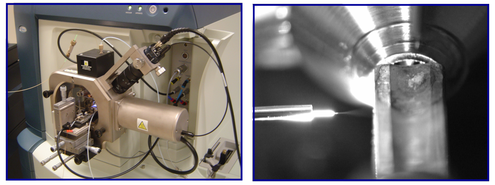

Figure 3. NanoLockSpray source - A NanoLockSpray source equipped with a CCD camera mounted on a Q-Tof Premier (left). The camera illustrates a uniform spray emanating from the emitter (right).

RESULTS AND DISCUSSION

Tip Regeneration

Although sensitive, current industry standard pulled-tip or tapered-tip emitters suffer from frequent clogging and eventually degrade. Unlike pulled tip emitters, if unstable spray is observed or if the emitter becomes obstructed the tip may quickly be regenerated. A diamond fused silica cleaving tool can be used to cut a small portion of the proximal end of the emitter leaving a fresh tip. The 1 cm length emitter may allow for up to 3 to 5 cuts.

The nanospray sprayer is designed to facilitate quick tip regeneration minimizing possible down time (shown below).

Regeneration procedure for nanoflow source:

1. The XYZ stage is transferred away from the inlet, nanospray is potential interrupted (Figure 4).
2. The adjustable stop on the sprayer is moved forward (Figure 4, left) exposing a length of the emitter.
3. A small portion (1-2 mm) of the emitter is cleaved (Figure, 4 right).
4. The emitter is retracted so that 1 mm of the tip extends beyond the nebulizer sheath by moving the adjustable stop.
5. The XYZ stage is repositioned and the nanospray potential is restored.

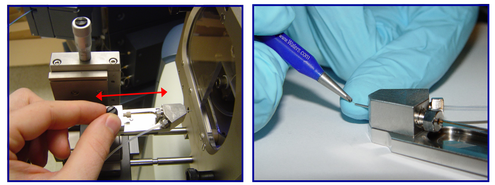

Figure 4. Regenerating an emitter - The adjustable stop on the nanospray sprayer used to expose the tip of the emitter from the nebulizer sheath (left). Once exposed the emitter tip can be cut/regenerated with a diamond cleaving tool (right).

An infusion of 150 fmol/µl of glufibrinopeptide (Glu-Fib) at 400 nl/min was interrupted 4 separate times to test the reproducibility of an emitter regeneration (Figure 5). After each regeneration the sprayer was properly repositioned and the signal intensity was restored. The average time for each cleavage was 30 seconds.

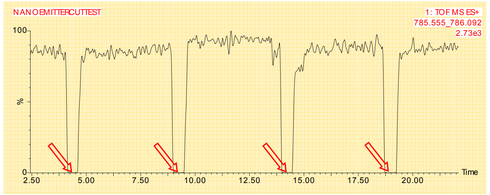

Figure 5. Regenerating an emitter - SIC of 785.843, the $(M+2H)^{+2}$ ion of Glu-Fib. The red arrows indicate the points at which the tip was regenerated.

Spray Stability and Sensitivity

Signal stability is of critical importance in any nanoLC based application. A solution of Glu-Fib (30% ACN, 0.1% formic acid) was infused into a Q-Tof micro via a Harvard syringe pump to demonstrate the spray stability of the emitter over an extended period of time. Tests were performed in excess of 100 minutes (Figure 6).

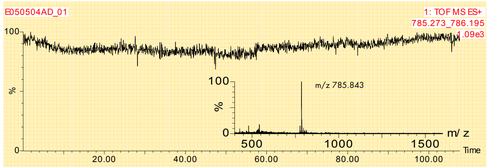

Figure 6. Nanospray stability - SIC of 785.843, the $(M+2H)^{+2}$ ion of Glu-Fib infused at 350 nl/min.

A complex mixture of 4 tryptically digested proteins (Enolase, Bovine Serum Albumin (BSA), alcohol dehydrogenase and phospohorylase B) was implemented to study spray stability of the emitter across a gradient. The gradient was delivered by a nanoACQUITY UPLC over a 30 minute period. Ten injections of 50 fmol were performed on a 75 µm x 10 cm, 1.7 µm BEH C18 nanoAcquity column with a 180 µm x 20 mm, 5 µm Symmetry® C18 trap column at a flow rate of 400 nl/min. The overlay of the 10 chromatograms (Figure 7), illustrates the reproducable ion intensity and stable spray over varying solvent conditions. The average peak area RSD for the10 injections, based on 10 peptides, was 3.28. The symmetrical peak shape and minimal peak width (median of 6.0 sec, 4σ) demonstrates the effective union between the emitter and the column.

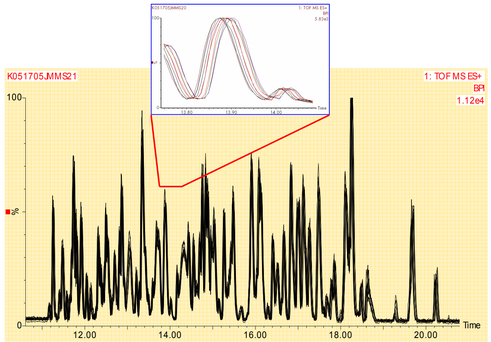

Figure 7. Nanospray stability - Overlay of 10 chromatograms derived from a tryptic digest of 4 proteins injected at a concentration of 50 fmol.

CONCLUSIONS

- A novel nanoelectrospray emitter has been designed to be sensitive and robust. Unlike common pulled fused silica emitters which frequently need to be replaced, the emitter may be regenerated multiple times allowing for an extended lifetime.
- A nanospray sprayer designed to incorporate the new emitters on a commercially available MS platform allows for tip regeneration/replacement, without removal from the ion source, in under 30 seconds.
- The emitter/sprayer combination has shown to provide a stable spray in both isocratic and gradient conditions. The diamond polished distal end of the emitter provides a true emitter/column union resulting minimal band broadening and exceptional sensitivity.

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