Improved Nanospray Emitter Coatings for Nanospray LC-MS

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The coupling of electrospray ionization (ESI) and mass spectrometry (MS) has had a dramatic impact on proteomics as it has developed into one of the leading techniques in the analysis of large biomolecules. Recent advances have emphasized increased sensitivity and complex mixture analysis by coupling nanobore chromatography columns with nanospray needles fabricated from fused silica tubing. In one particularly common configuration, fused silica tips are layered with a conductive coating to provide voltage contact at the end of the tip. Such coatings are susceptible to electrical discharges, electrochemical reactions and poor mechanical/chemical adhesion to the fused silica substrate. Novel tip coatings were designed and explored with the ultimate goal of increasing nanospray emitter performance.

Online electrospray fused silica needles with i.d.'s ranging from 10 to 30 µm and offline glass needles with i.d.'s of 2 and 4 µm, were coated by a variety of vacuum and conformal techniques. Metal (Au, Pt, Ti) and inorganic (SiO, C) multi-layer films were deposited by either vacuum evaporation or vacuum sputtering. Several tips were overcoated with an organic polymer (polyimide) coating applied by either dip coating or spray coating. The electrospray emitters were tested on a benchtop spray apparatus using a high voltage power supply (Stanford Research Systems, Inc). Tip and coating morphology and ESI plume geometry was monitored with a high resolution video imaging system. Mobile phase (50% MeOH, Water, 2% Acetic Acid) was delivered at a flow of 150 nl/min with a high precision syringe pump (Harvard Apparatus). Emitter performance was also evaluated with a Thermo Finnigan LCQ^{Deca} ion trap mass spectrometer equipped with a PicoView^{TM} ion source.

Historically, gold has been a common conductive material used in emitter fabrication. Although excellent electrochemical properties make the coating a good choice, poor chemical adhesion to the silica substrate results in an observed rapid delamination of the metal from the tip of the emitter in less than 24 hours. Metals with similar electrochemical performance such at platinum were studied as an alternative. To supplement the metal coating several overcoats of SiO were placed on the metallic surface to insulate the material and discourage delinimation. Platinum applied via sputtering in a reactive atmosphere to dramatically improve adhesion to the silica substrate, showed to be particularly promising. When combined with the SiO overlayer, also under reactive conditions, such tips were capable of performing ESI for hundreds of hours (800+) of continuous operation with MeOH/Water/Acetic acid mobile phase. Figure 1 shows a mass spectrum of Ubiquitin acquired with a multilayered SiO/Pt coated electrospray emitter after over 830 hours of continual spraying on the benchtop apparatus. The platinum coating added no detectable background to the MS signal when compared with uncoated tips making them suitable for use with complex peptide mixtures. The platinum coating also withstood more aggressive tests such as sonication in a solution of acetonitrile with 5% formic acid.

Several composite coatings techniques were also studied including those in which a conductive material was insulated in a polymer matrix (polyimide). The fabrication of tips with these methods is difficult to reproduce, the lifetime experiments appear encouraging. Initial MS experiments show the addition of a polymer layer does not create an appreciable increase in a background signal.

A second benchtop spray apparatus was constructed to test the coatings resistance to electrical discharge. The emitters, positioned 1 mm from a counter electrode, were allowed to arc at a voltage of 5 kV. The Pt/SiO coated tips were capable of operating after severe arcing events which left simple gold coated tips inoperable.

The impact of the resistivity of the emitter coating was studied with the use of ultra-high resistive coatings fabricated by sputter deposition. Two coatings of interest include titanium and diamond-like-carbon (DLC). Even with a film resistance of greater than 2000 meg-ohms, ESI is routinely achievable, suggesting that a wider range of materials could prove suitable for emitter fabrication. Although the two

coatings studied here had a very short lifetime (less than 10 hours) such films may offer the advantage of a built-in current limiting resistor to improve spray stability.

Emitter coatings were also tested with an offline or static nanospray apparatus. A reactive coated platinum emitter was filled with 5 μl of 10 μM Angiotensin I solution while the total ion chromatogram (TIC) was obtained by a Thermo Finnigan LCQ^{Deca} (Figure 2). After approximately 60 minutes a drop in intensity was observed due to the emitter running out of sample. The emitter was then reloaded and allowed to run for over 270 minutes (equating to a flow of 70 nl/min). The emitter was capable of electrospraying for additional time but was stopped due to time restraints. Static nanospray emitter lifetime is not limited by its coating but to physical constraints such as clogging.

The platinum coated tips have proven suitable for the direct coupling of the nanobore chromatography of complex peptide mixtures, yielding operation times that enable 24 hour operation suitable for use with autosamplers. Additional novel coatings, including highly resistive materials, are currently being explored to produce a variety of long-lasting electrospray emitters.

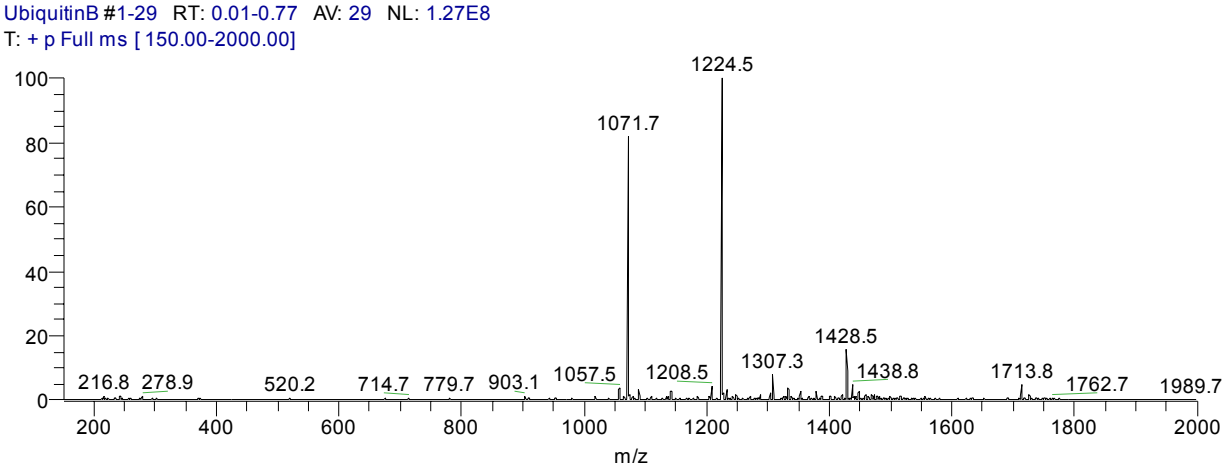

Figure 1 – Mass spectrum of a 10 μM Ubiquitin solution produced with a Pt/SiO multilayered emitter with over 830 hours of service. The mass spectrum was aquired by a Thermo Finnigan LCQ^{Deca} equipped with a PicoView nanospray source.

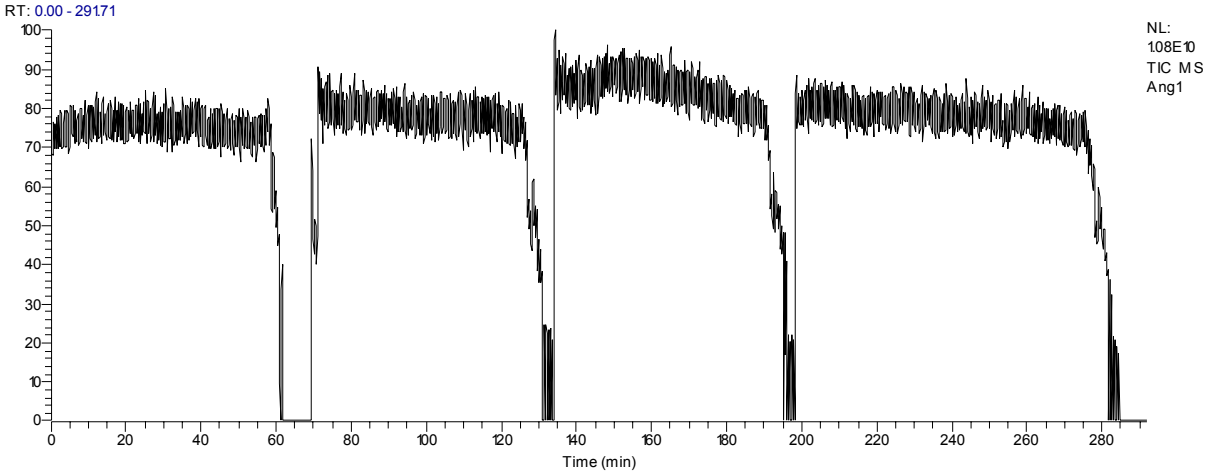

Figure 2 - Total ion chromatogram of Angiotensin I from an offline nanospray emitter with a reactive platinum coating.