

LOW-COST MICROFLUIDIC EMITTERS FOR NANO-ELECTROSPRAY IONIZATION-MASS SPECTROMETRY

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ABSTRACT

We report a new microfluidic emitter for nano-electrospray ionization mass spectrometry (nanoESI-MS), made from polyimide tape. In contrast to the conventional pulled glass capillary emitters typically used for nanoESI-MS, these new polyimide emitters are simple and inexpensive to make, and are disposable after a single use. Thus, sample contamination and clogging are never a problem when analyzing different samples. The new emitters have comparable performance to pulled glass emitters, and are applicable to a variety of sample types and volumes, with the advantages of low cost, ease of use, and disposability.

KEYWORDS: Mass Spectrometry, Nano-Electrospray Ionization, Low-Cost, Disposability

INTRODUCTION

Since its coupling with mass spectrometry (MS) more than 25 years ago¹, electrospray ionization (ESI) has become an established technique for the analysis of a wide range of molecules, and has found its niche in the analysis of large biomolecules in the solution phase.² ESI has transformed MS from a specialized technique studied by experts to a readily accessible tool used in laboratories worldwide. The development of nanoESI-MS has further extended the utility of ESI-MS, permitting the analysis of small volume samples with enhanced sensitivity.^{3,4} Traditional nanoESI-MS experiments use pulled-glass capillary emitters, which are expensive to purchase and require specialized instruments and training to fabricate in-house. Furthermore, these emitters are frustrating to work with, suffering from problems including clogging, sample contamination, and irreproducible spray stability. Myriad alternatives to pulled glass capillary emitters have been described in the literature, many of which are formed by microfabrication and are often coupled with microfluidics.⁵ Although these emitters have found utility in academic laboratories and constitute an active field of research, the majority suffer from the significant costs incurred in microfabrication, which inhibits widespread use.

Here we report a new low-cost emitter for nanoESI-MS, formed by folding a thin film of polyimide tape into a cone with a micron-sized orifice at the apex. These new emitters are easy to make and do not require any specialized equipment to fabricate or use. The low cost of these emitters allows for single use, such that sample carry-over and clogging are not a problem. The new emitters show comparable performance to pulled glass emitters and are applicable to a variety of sample types and volumes. These emitters are a low-cost alternative to glass capillary emitters for applications involving single use direct analysis by nanoESI-MS.

EXPERIMENTAL

Each emitter was formed by folding a piece of polyimide tape (5 cm x 5 cm) into a cone shape such that there was a small opening at the apex of the cone (Figure 1a). The diameter of the orifice from which the electrospray emanates is controlled by how tightly the tape is folded, and can be tuned from ~35 μm by inducing significant torque when folding, to greater than 200 μm if loosely formed into a cone. Smaller orifice diameters result in smaller charged droplets in the electrospray, thus increasing detection sensitivity. Therefore, polyimide emitters were generally folded with small orifice diameters, in the range of 35-55 μm (Figure 1a inset).

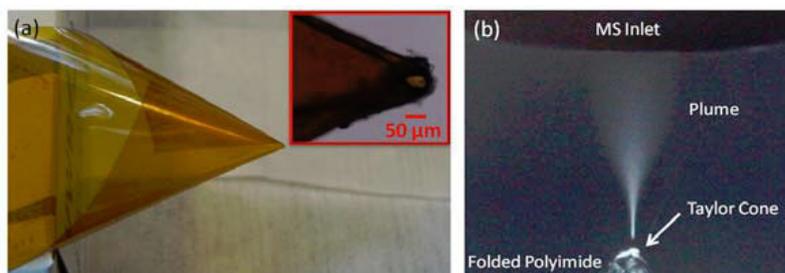


Figure 1: (a) Picture of a polyimide nanoESI emitter formed by folding a piece of polyimide tape into a cone (main panel), and picture at 10x magnification showing the orifice from which the electrospray is formed (inset). (b) Taylor cone and electrospray generated by applying +2.40 kV to a solution of 4:1 acetonitrile (ACN)/water (H_2O) (v/v) contained in the tip of a folded nanoESI emitter. The spray voltage is applied via a platinum wire in contact with the solution to be sprayed, and the tip is positioned ~3 mm from the grounded inlet of the mass spectrometer.

Folded polyimide emitters were positioned ~3 mm from the grounded inlet of the mass spectrometer. Solutions to be analyzed were pipetted into the tip of the emitter through the rear (wide) side of the cone. For most emitters, spray voltage was applied via a platinum wire (100 μm diameter) inserted in the rear of the emitter, making contact with the solution. The Taylor cone and electrospray generated from an emitter with a ~50 μm orifice operated in this mode is shown

in Figure 1b. Alternatively, some emitters were formed with integrated wires for applying spray voltage. 150 μm gold wires were patterned using conventional microfabrication techniques (metal deposition, photolithography, and wet etching) on polyimide substrates, which were then folded into the desired cone shape, ensuring the wire was positioned at the orifice to make contact with the solution being sprayed.

All experiments were performed using an LTQ linear ion trap mass spectrometer. Applied potentials were varied in the range of +2-3 kV for each experiment performed with a unique polyimide emitter to optimize the observed signal. Parameters including capillary voltage, tube lens voltage, and others were also varied for each experiment to provide optimum signal. In the case of spectra collected in negative ion mode, the applied potential was -3.2 kV. Performance was evaluated based on total ion count (TIC) scans. For comparison, pulled glass capillary emitters were also evaluated. In such experiments, analytes were infused at a flow rate of 0.8 $\mu\text{L}/\text{min}$, and the driving potential was varied in the range of +1.3- 1.8 kV.

RESULTS AND DISCUSSION

The new polyimide emitters are simple to prepare and take only a few seconds to fold. The process does not require any specialized equipment, and there is little skill or training involved in forming small diameter orifice emitters. The cost of a single emitter formed from a 5 cm x 5 cm piece of polyimide tape is only ~\$0.07 in materials, and less than one minute of time. In contrast, pulled glass capillary emitters require a costly (\$5000-10 000) capillary puller to fabricate in-house, or can be purchased at a significant cost of \$10-20 each. If fabricating in-house, capillary pulling is a process that requires significant time, practice, and skill to master, and has a low success-rate in pulling usable emitters. Since the new polyimide emitters are so inexpensive, they are ideal for single use applications. When applied in the single-use format, the problems of contamination or carry-over from previous analyses, and clogging of the tip when switching between different samples are eliminated.

An important factor when assessing the performance of an electrospray method is spray stability. A stable spray ensures low variation in the number of ions formed, with spectral features (background, base peak and other mass peaks) remaining relatively constant over time. The spray stability of the polyimide emitter was characterized by sampling a solution of the MS calibration standard Ultramark 1621 and evaluating the resulting total ion count (TIC) scan. As seen in Figure 2, the spray generated from a polyimide emitter shows comparable stability to a pulled glass capillary emitter over greater than 12 minutes, as evidenced in the low variation in the TIC scans for both emitters.

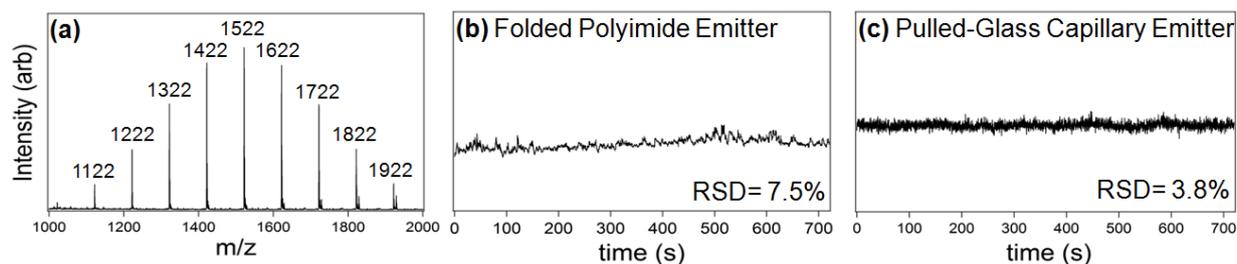


Figure 2: (a) Mass spectrum of Ultramark 1621 generated using a folded polyimide nanoESI emitter, and (b) corresponding TIC trace. (c) TIC trace for a pulled glass capillary emitter. The infused solution was Ultramark 1621 (1 μM in 2:1:1 ACN/methanol (MeOH)/H₂O, 0.1% acetic acid (v/v/v)). Applied potential was +2.4 kV for (a) and (b), and +1.3 kV for (c). A 50 μL droplet was sampled in (a) and (b), while the solution was infused at a flow rate of 0.8 $\mu\text{L}/\text{min}$ for (c).

The capacity of the folded emitters to ionize samples for subsequent mass analysis was evaluated for a variety of analytes, ranging from synthetic polymers, to small molecules, peptides, and proteins. A spectrum of the synthetic polymer Ultramark 1621 is shown in Figure 2a. A spectrum of the amino acid methionine is shown in Figure 3a, clearly displaying low background signal and no interfering peaks in the low mass range. A spectrum of the peptide leucine enkephalin is seen in Figure 3b. A spectrum of the protein myoglobin is shown in Figure 3c; multiple charge states of the protein and the heme monomer are clearly distinguishable. Figure 3d shows a spectrum of the hormone estradiol E2 collected in negative ion mode; the [M-H]⁻ molecular ion peak is easily observed. This spectrum collected in negative ion mode is particularly important in evaluating the performance and versatility of the folded emitters, as negative-ion electrospray ionization is generally difficult (even when working with pulled glass capillary emitters) because of the propensity of negative potentials for forming corona discharge.

An alternative method to supply the driving potential to polyimide emitters is via thin wire patterned directly on the polyimide tape prior to folding for direct application of spray voltage. An example of such an emitter is presented in Figure 4a. A spectrum of Ultramark 1621 collected using an emitter with a microfabricated gold wire is shown in Figure 4b; the spectrum is comparable to spectra collected using both an unpatterned polyimide emitter and a pulled glass capillary emitter (Figure 2). Although emitters with the integrated wire require additional preparation, fabrication is fairly simple (consisting of a single photolithography step) and eliminates the need for an external wire to be interfaced to the system. Fabrication of gold wires directly on polyimide emitters presents future possibilities for coupling to microfluidics, with the advantage of relatively simple fabrication when compared to other microfabricated nanoESI emitters.

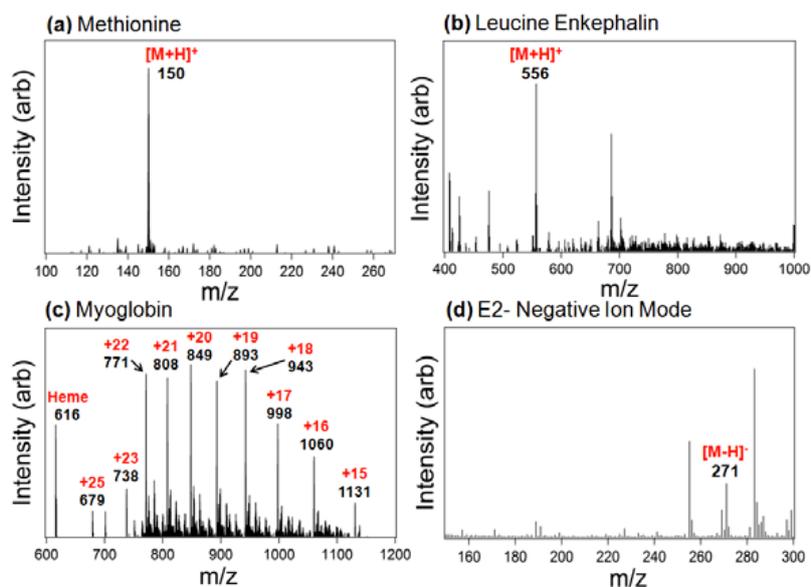


Figure 3: Mass spectra generated using polyimide nanoESI emitters. (a) methionine ($1 \mu\text{M}$ in 7:3 ACN/ H_2O containing 0.1% trifluoroacetic acid (v/v)); (b) leucine enkephalin ($1 \mu\text{M}$ in 7:3 ACN/ H_2O containing 0.01% formic acid (FA) (v/v)); (c) myoglobin ($3 \mu\text{M}$ in 1:1 MeOH/ H_2O containing 0.1% FA (v/v)); (d) Estradiol E2 ($1 \mu\text{M}$ in 4:1 ACN/ H_2O (v/v)) collected in negative ion mode. Capillary temperature was set at 200°C for all analyses.

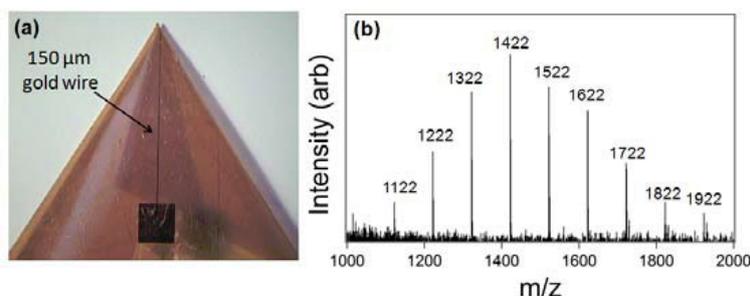


Figure 4: Polyimide nanoESI emitter with a microfabricated gold wire for application of spray voltage. (a) Image of the emitter. The gold wire runs directly to the orifice to ensure contact with a solution placed in the tip. (b) Mass spectrum of Ultramark 1621 obtained using a polyimide emitter with a microfabricated gold wire.

CONCLUSION

We present a new emitter for nanoESI-MS, employing a piece of polyimide tape folded into a cone. When compared to pulled glass capillary emitters, the new emitters are simple to make and much less expensive, costing only $\sim\$0.07$ in materials and a few seconds in preparation time. The new emitters are applicable for the analysis of a wide range of analytes, can be used in both positive and negative ion mode, and show comparable performance and spray stability to pulled glass emitters over extended periods of time. Folded polyimide emitters show great promise as a low-cost alternative for applications involving single use direct analysis by nanoESI-MS.

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