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Folded emitters for nanoelectrospray ionization mass spectrometry

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Electrospray ionization (ESI) has revolutionized mass spectrometry (MS), providing a facile method for the ionization of macromolecules for analysis by mass. The development of nanoESI-MS has further extended the utility of ESI-MS, permitting the analysis of small-volume samples with enhanced sensitivity over conventional ESI-MS. 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 suffer from problems including clogging, sample contamination, and irreproducible spray stability. Here, we report a new emitter for nanoESI-MS, made by folding small pieces of polyimide tape. In comparison with conventional pulled-glass capillary emitters, the new emitters are inexpensive and simple to make. Their low cost makes them disposable after a single use, such that sample contamination or clogging is never a problem. Emitter performance has been evaluated for diverse analytes encompassing a large mass range, including small molecules, peptides, proteins, and synthetic polymers. In all cases, the performance is similar to that of pulled-glass capillary emitters, with the advantages of low cost, ease of use, and disposability.

Since its coupling with mass spectrometry (MS) more than 25 years ago,[1] 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 is gentle enough to overcome the propensity of such molecules to fragment upon ionization prior to MS analysis, providing mass and structural information.[3] In short, ESI has transformed MS from a specialized technique studied by experts to a readily accessible tool used in laboratories worldwide.

In traditional ESI-MS, solutions are pumped at μL/min flow rates through silica or metal capillaries positioned close to the inlet of a mass spectrometer, and a nebulizing gas aids in electrospray formation.[4] This format is ideal for coupling with separations, including high-performance liquid chromatography (HPLC) and capillary electrophoresis; however, ESI-MS is limited in its ability to accommodate small-volume samples. This limitation was overcome by the development of nanoESI-MS,[5,6] a related technique in which samples are sprayed at nL/min flow rates from capillaries with tapered, narrow-bore orifices placed within millimeters of the inlet of a mass spectrometer.[7] Electrospray is generated simply by the application of high potential to the solution, eliminating the need for nebulizing gas. In addition, the lower solution flow rates used in nanoESI result in smaller droplets in the electrospray, each of which requires fewer droplet fissions and less solvent evaporation to generate single gas-phase ions. Consequently, a greater number of analyte molecules are available for ionization, such that nanoESI has greater ionization efficiency (and hence sensitivity) than traditional ESI.[8,9]

In nanoESI, analytes are typically sprayed from glass capillary emitters with 1–30 μm diameter orifices. These are often formed by mechanically pulling the end of a glass capillary while heating, forming a sharpened tip. Such emitters can be fabricated in-house using a capillary puller, but this process requires time, training, and significant skill. In fact, capillary pulling is still regarded as more art than science, with low success rates in pulling capillaries with desirable small diameter orifices. Emitters are also available commercially, albeit at a significant cost of approximately $10–20 per emitter.[10] In addition, pulled-glass capillary emitters are frustrating to work with, suffering from problems such as clogging, sample contamination, and irreproducible spray stability.[11] Clogging and contamination are especially likely to take place when switching between different samples for analysis, and in most cases the only solution is to dispose of the old emitter and replace it with a new one. For labs that run many samples each day, the costs associated with these emitters (monetary if

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purchased, and time and skill if fabricating in-house) is significant. Pulled-glass emitters also suffer from high tip-to-tip variability, and since nanoESI mass spectra show strong dependence on the emitter used, the reproducibility of spectra collected using different emitters is low.[9] Although nanoESI has enormous potential as an analytical tool, these problems have inhibited the technique from being adopted universally.

While the gold-standard for nanoESI emitters is pulled-glass capillaries, there are myriad alternatives described in the literature. Many such alternative emitters are formed by microfabrication and are often coupled with microfluidics.[12–19] Although these emitters have found utility in academic laboratories and constitute an active field of research, they often require laborious, time-consuming cleanroom fabrication (with noted exceptions[19]), and are thus typically limited to laboratories with access to microfabrication equipment and expertise. Therefore, such emitters are not likely to become a viable commercial alternative for nanoESI-MS. As a counter-point, an example of a success in this area is the microfabricated nanoESI interface developed by Yin *et al.*[20] and commercialized by Agilent Technologies as the HPLC Chip^{TM}.[21] This device is formed by laser ablation of polyimide substrates and is capable of separations and MS analysis with detection characteristics similar to those obtained with conventional techniques. This device is a competitor to pulled glass capillary emitters, but the significant cost (~$500 each) is a barrier to wide-spread adoption.

There are few examples of low-cost ESI emitters that do not require microfabrication. One creative method comprises spraying from the orifice of a plastic pipette tip, where driving potential is applied via a wire inserted in the rear of the pipette tip.[22] This technique serves as a disposable, inexpensive alternative to pulled-glass capillary emitters, but suffers the drawback of the relatively large diameter of the orifice (~300 μm). These orifices are an order of magnitude larger than conventional nanoESI pulled-glass capillary emitters, and this translates to larger charged droplets in the electrospray, and hence a sacrifice in detection sensitivity. Another example of a low-cost emitter was recently reported by Cooks and co-workers.[23,24] Their technique employs a small piece of paper cut to a sharp point; sample is transported to the tip by capillary action, and voltage is applied directly to the paper. This method has utility for the analysis of biological samples, but disadvantages include the high spray voltages required (4.5–5.0 kV in positive ion mode) and the limited spray stability over time (less than 2 min for a 10 μL sample).

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

EXPERIMENTAL

Materials

Polyimide tape (87.5 μm thickness; 50 μm polyimide with 37.5 μm silicone-based adhesive) was purchased from Argon Masking, Inc. (Monrovia, CA, USA). Unless indicated, all reagents were from Sigma Aldrich (Oakville, ON, Canada). Ultramark 1621 was from Thermo Fisher Scientific (Ottawa, ON, Canada). Hexamethyldisilazane (HMDS) was from Shin-Etsu MicroSi (Phoenix, AZ, USA), Shipley S1811 photoresist and MF321 developer were from Rohm and Haas (Marlborough, MA, USA), chromium and gold were from Kurt J. Lesker (Toronto, ON, Canada), and CR-4 chromium etchant was from Cyantek (Fremont, CA, USA). HPLC-grade solvents and deionized water (diH_2O) with a resistivity of 18 MΩ · cm at 25°C were used in all experiments.

For MS, Ultramark 1621 was diluted in 2:1:1 acetonitrile (ACN)/methanol (MeOH)/diH_2O containing 0.1% acetic acid (v/v/v) to a 1 μM final concentration. Methionine was dissolved in diH_2O and diluted in 7:3 ACN/diH_2O containing 0.1% trifluoroacetic acid (TFA) (v/v) to a final concentration of 1 μM. Leucine enkephalin was dissolved in diH_2O and diluted in 7:3 ACN/diH_2O containing 0.01% formic acid (FA) (v/v) to a final concentration of 1 μM. Myoglobin was dissolved in diH_2O and diluted in 1:1 MeOH/diH_2O containing 0.1% FA (v/v) to a final concentration of 3 μM. Estradiol (E2) was dissolved in diH_2O and diluted in 4:1 ACN/diH_2O (v/v) to a final concentration of 1 μM. Angiotensin I was dissolved in diH_2O and diluted to a final concentration of 1 μM in 4:1 ACN/diH_2O containing 0.1% TFA (v/v).

Preparation of emitters

Each emitter was formed in a conventional laboratory (i.e., not a cleanroom) by folding a piece of polyimide tape (5 cm × 5 cm) into a cone shape such that there was a small opening at the apex of the cone. The size of the opening was controlled by how tightly the tape was folded, resulting in orifices ~35–60 μm in diameter. The adhesive backing of the polyimide tape facilitated quick and efficient folding of emitters, and was useful for holding the cone in the desired shape. Initial experiments using adhesive-free polyimide film (using Scotch tape to hold the cone in place) were unsuccessful in maintaining desired small diameter orifices. Emitters were rinsed three times with diH_2O or another appropriate solvent before use. Emitter orifices were imaged and average orifice diameters were found using an upright microscope (Leica DM2000; Leica Microsystems Inc., Richmond Hill, ON, Canada).

Fabrication of integrated wires

Some emitters were formed with integrated wires for applying spray voltage in a class 1000 cleanroom facility at the Emerging Communications Technology Institute (ECTI) in the University of Toronto. For these devices, prior to being folded (as described above), substrates were patterned by photolithography to form a 150 μm wide × 4 cm cm long wire running from the back opening of the emitter. Photolithography was implemented using conventional methods: briefly, sections of polyimide tape were first adhered to glass microscope slides and then primed for metal adhesion by reactive ion etching (RIE, 150 W, 98 sccm O_2,

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100 mTorr, 30 s) in a Trion Phantom etcher (Clearwater, FL, USA) before deposition of a 75 nm seed layer of chromium and 200 nm layer of gold by electron beam evaporation (Auto 306; Edwards, Wilmington, MA, USA). Substrates were cleaned with acetone and methanol, baked on a hot plate (95°C, 2 min), primed with HMDS, and spin-coated with S1811 (3000 rpm, 30 s). Substrates were pre-baked on a hot plate (95°C, 2 min) and photolithographically patterned by UV exposure (365 nm, 35 mW/cm^2, 5 s) through a photomask (Pacific Arts and Designs, Markham, ON, Canada) using a MA6 mask aligner (Karl-Süss, Garching, Germany). Substrates were developed in MF321 (2 min) and post-baked on a hot plate (105°C, 1 min) before being immersed in gold etchant (1 min) and then in CR-4 (2 min). Finally, the remaining photoresist was removed in AZ300T (10 min). After fabrication, substrates were removed from the glass microscope slides, cut with a scalpel to ensure that the wire ran to the edge of the tape, and manually folded into the desired cone shape, taking care that the wire was positioned at the orifice to make contact with the solution being sprayed.

MS analysis

All experiments were performed using an LTQ linear ion trap mass spectrometer (Thermo Scientific, Waltham, MA, USA). Folded polyimide emitters were placed on a *xyz*-positioning stage (Parker Automation, Cleveland, OH, USA) ~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. Alternatively, for emitters with integrated metal contacts, a driving potential was applied to the microfabricated wire using an alligator clip. Unless otherwise indicated, the transfer capillary temperature was 200°C. 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. The capillary voltage and tube lens voltage were 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. Emitter performance was evaluated based on total ion count (TIC) scans by injection of a 1 μM solution of Ultramark 1621 (2:1:1 ACN/MeOH/diH_2O containing 0.1% acetic acid (AA) (v/v/v)). MS/MS analysis of angiotensin I was performed with a collision energy of 30 eV. The spectra presented were obtained by averaging 10–50 acquisitions (at a rate of 1–6 acquisitions/s) and are representative of separate analyses performed per unique experimental condition. Images of the electrospray were collected using a CCD camera positioned perpendicular to emitters placed ~3 mm from the grounded inlet of the mass spectrometer.

For comparison, pulled-glass capillary emitters (FS360-50-30-N-20, 360 μm o.d. capillary, tapering to a 50 μm o.d./30 μm i.d. tip; New Objective, Woburn, MA, USA) were also evaluated. In such experiments, analytes were infused at a flow rate of 0.8 μL/min, and the driving potential was varied in the range of + 1.3–1.8 kV.

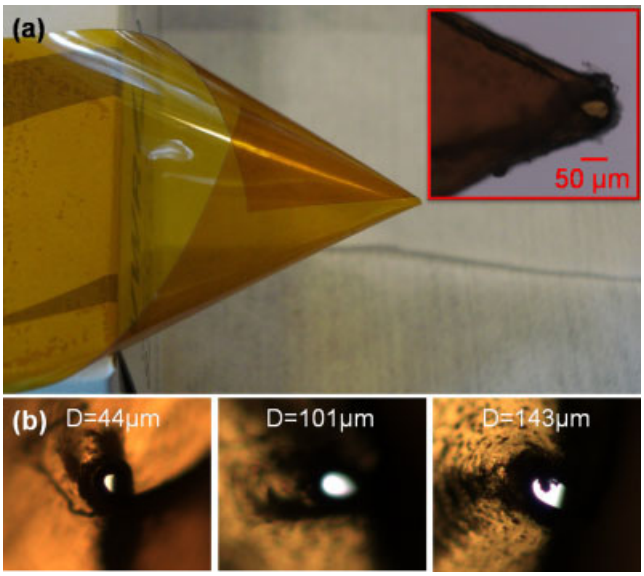

Figure 1. Folded polyimide emitters. (a) Picture of a nanoESI emitter formed by folding a 5 cm × 5 cm piece of polyimide tape into a cone (main panel), and picture at 10× magnification showing the orifice from which the electrospray is formed (inset). (b) Images of nanoESI emitters folded with different orifice diameters. Diameters reported are elliptical averages, taken as the average when measuring both the width and the height of the emitter orifice.

RESULTS AND DISCUSSION

Emitter preparation and use

The new emitters described here are formed by folding a piece of commercially available polyimide tape into a cone (Fig. 1(a)). Polyimide was chosen as a substrate for this application because of its chemical and mechanical stability[25] and modest expense. 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 (Fig. 1(b)). For the applications presented here, emitters were folded with orifice diameters in the range of 35–60 μm (Fig. 1(a), inset). Orifice diameter measurements of 10 folded emitters had an average orifice diameter of (52.6 ± 6.5) μm (mean ± standard deviation).

Folded 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 × 5 cm piece of polyimide tape is only ~$0.07 in materials, and requires less than 1 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 for $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. Although formation of a single pulled-glass emitter is inexpensive and fast, in practice, several flawed emitters are often formed in the pursuit of one usable emitter, requiring extra time and resources. Folded polyimide emitters present an attractive alternative to pulled glass emitters from both a cost and resource perspective.

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Since folded emitters are so inexpensive, they are ideal for single use applications. When applied in 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.

When using the new emitters, samples are pipetted directly into the rear aperture of the folded polyimide tape, and electrospray is generated by application of high voltage to the solution via a platinum wire placed in contact with the solution. One of the advantages of folded emitters is the potential to accommodate a wide range of sample volumes, such that small-volume samples do not need to be diluted, and larger volumes can be sampled for extended periods of time, eliminating sample waste. Since samples are pipetted directly into emitters, no sample is wasted in pre-treatment of the system, and total sample volumes less than 10 μL can be used to generate electrospray and collect mass spectra. No specialized equipment is needed to supply liquid to folded emitters, as ionization occurs directly from static droplets. In contrast, traditional ESI requires a syringe pump and associated fittings for sample infusion to the emitter, further complicating the system.

Spray performance

The electrospray generated from two different folded polyimide emitters is shown in Fig. 2. In Fig. 2(a), the Taylor cone and electrospray from an emitter with a ~50 μm orifice is shown, while the same is shown in Fig. 2(b) for an emitter with an orifice diameter of ~150 μm. It is clear that the larger orifice diameter results in a larger Taylor cone. Since the Taylor cone is formed before electrospray, larger Taylor cones increase sample consumption rates, diminishing the ability to collect data from small-volume samples over longer periods of time. Although flow rate cannot be used in this context to quantify sample consumption, we can estimate a sample consumption rate based on the observation of electrospray and the collection of mass data over time. For folded emitters with orifices 35–55 μm in diameter, the sample consumption is approximately 1000 nL/min, and this increases with orifice diameter, to more than 2 μL/min for emitters with ~150 μm orifices.

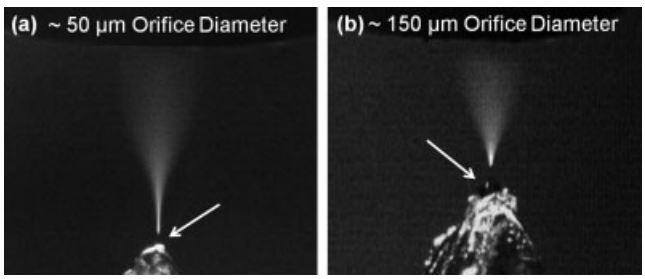

Figure 2. (a) Taylor cone and electrospray generated from a folded emitter with ~50 μm orifice diameter at an applied voltage of +2.4 kV. (b) Taylor cone and electrospray generated from a folded emitter with ~150 μm orifice diameter with an applied voltage of +3.1 kV. In both cases the solution sprayed is 4:1 ACN/diH_2O (v/v). Spray voltage is applied via a platinum wire in contact with the solution to be sprayed. Emitters are positioned ~3 mm from the grounded inlet of the mass spectrometer. The Taylor cone is much larger for the larger orifice emitter, thus increasing sample consumption rates when compared to emitters with smaller orifice diameters.

The solvent consumption rates of the new emitters (above) pose an interesting question related to nomenclature – namely, is this method truly ''nano''-electrospray ionization? The flow rates are higher than typically expected for nanoESI (which usually employs flow rates of 20–1000 nL/min), and are comparable with that of so-called microESI[26] (a relatively uncommon designation used for emitters that have intermediate size between nano- and conventional ESI), in which flow rates in the 0.2–3 μL/min range are used. In addition, the orifice diameters of the new folded emitters are larger than standard nanoESI pulled-glass capillary emitters. On the other hand, the new folded emitters do not require samples to be pumped to the emitter (liquids are sampled statically) and a nebulizing gas is not required, both features characteristic of nanoESI. Thus, the new folded emitters described here represent a hybrid between micro- and nanoESI, possessing characteristics of both. For brevity, we have used ''nanoESI'' in this manuscript.

Another 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 folded emitters was characterized by sampling a solution of the MS calibration standard Ultramark 1621 and evaluating the resulting TIC scan. As seen in Fig. 3, the spray generated from a folded emitter shows similar stability to that of a pulled-glass capillary emitter over greater than 12 min, as evidenced in the low variation in the TIC scans for both emitters. In addition, as shown, the emitters allow for collection of MS data with comparable signal intensities. Finally, spray stability was best for folded emitters using solvents containing at least 50% organic solvent (i.e., acetonitrile or methanol).

MS performance

The ability of the folded emitters to ionize samples for 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 Fig. 3(a). Ultramark is composed of a mixture of fluorinated phosphazenes, and is characterized by a series of intense singly charged peaks equally spaced by 100 *m/z* units.[27] It is a good benchmark for MS emitters, and is used as a high mass range (*m/z* 1000–2000) calibration standard for ESI sources. A spectrum of the amino acid methionine is shown in Fig. 4(a), clearly displaying low background signal and no interfering peaks in the low-mass range. A spectrum of the peptide leucine enkephalin is shown in Fig. 4(b). A spectrum of the protein myoglobin is shown in Fig. 4(c); multiple charge states of the protein, as well as the accompanying heme monomer, are clearly distinguishable. Figure 4(d) shows a spectrum of the hormone estradiol E2 collected in negative ion mode; the $[M–H]^-$ ion peak is easily observed. This spectrum is particularly important in evaluating the performance and versatility of the folded emitters, as negative ion ESI is generally difficult (even when working

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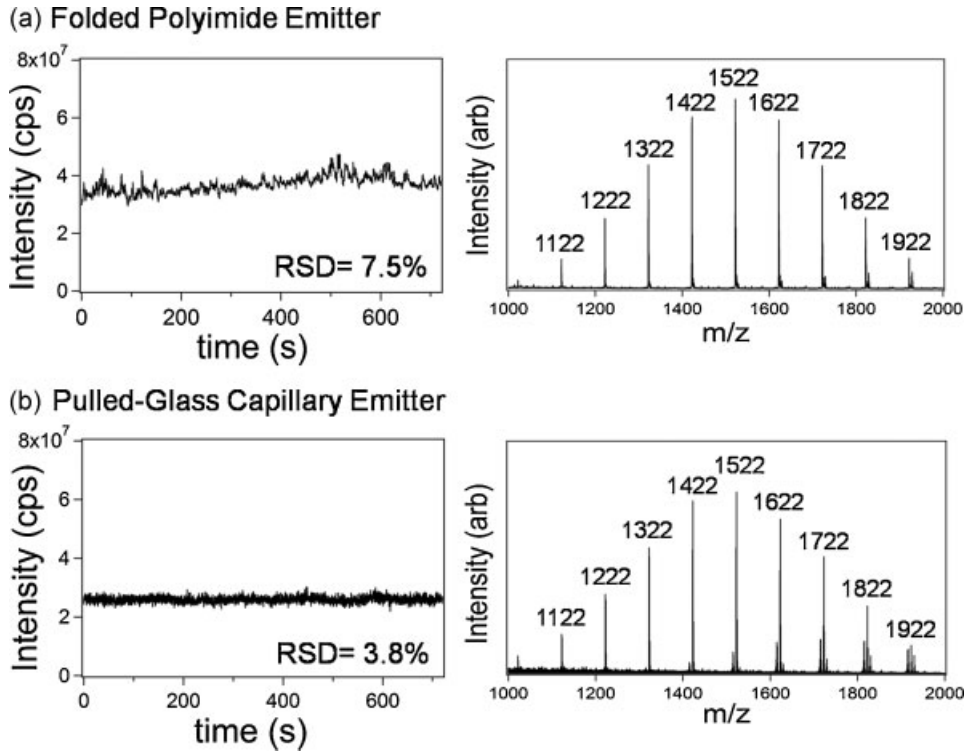

Figure 3. Total ion count (TIC) traces (left) and corresponding mass spectra (right) for (a) a folded polyimide emitter and (b) a pulled-glass capillary emitter. The infused solution was Ultramark 1621 (1 μM in 2:1:1 ACN/MeOH/diH_2O, 0.1% AA (v/v/v)). The applied potential was +2.4 kV for (a) and +1.3 kV for (b). A static 50 μL droplet was sampled in (a), while the solution was infused at a flow rate of 0.8 μL/min for (b). Capillary temperature was set at 200°C. The low variation in the ion count signal indicates a stable spray over more than 12 min for both emitters.

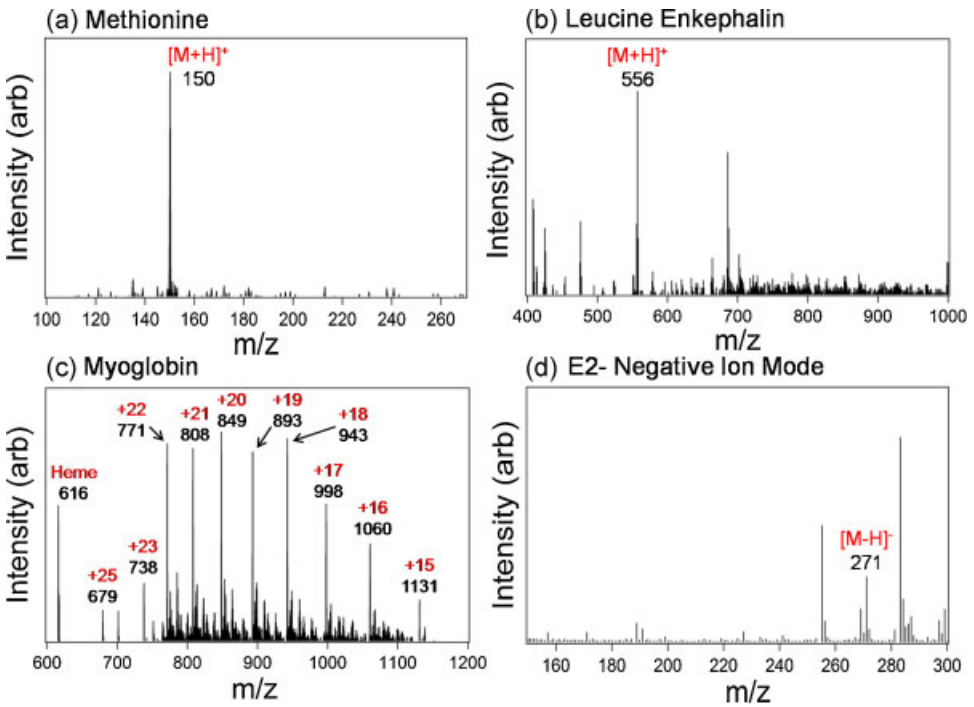

Figure 4. Mass spectra generated using folded polyimide emitters: (a) methionine (1 μM); (b) leucine enkephalin (1 μM); (c) myoglobin (3 μM); and (d) estradiol E2 (1 μM) collected in negative ion mode. All solutions were prepared as outlined in the Experimental section. Capillary temperature was set at 200°C for all analyses, and spray voltage and capillary voltage were varied for each unique analysis to optimize the observed signal.

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with pulled-glass capillary emitters) because of the propensity of negative potentials to form corona discharge.[28] Of note in all these spectra is that no background peaks from the polyimide substrate or silicone-based adhesive have been identified. The new emitters can be used to collect data over a wide mass range (*m*/z 100–2000) without any peaks from the adhesive or substrate interfering with the detection of the desired analyte.

Tandem mass spectrometry (MS/MS) coupled with collision-induced dissociation (CID) has become an important tool for analyte identification using MS, permitting unbiased identification of precursor ions based on unique product ions after CID.[29] MS/MS is used extensively for peptide sequencing, and is now the method of choice for proteome profiling.[30] To further demonstrate the utility of this new technique, a folded emitter was used to perform MS/MS analysis of angiotensin I. As shown in Fig. 5(a), two multiply charged precursor ions were present in the first mass selection. Isolation of the triply protonated precursor ion followed by CID led to the identification of several *b*- and *y*-ion peptide fragments in the second mass selection (Fig. 5(b) and inset).

Emitters with integrated wires

The applications of folded emitters described above employ a platinum wire inserted in the rear of the emitter to supply spray voltage to the solution. This method is attractive because of its simplicity – any competent researcher can form

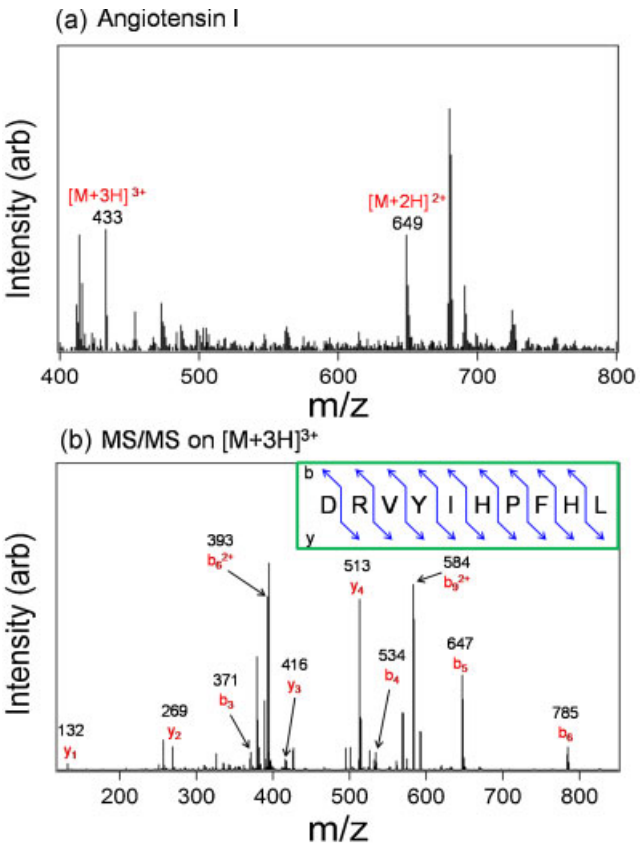

Figure 5. (a) Spectrum of angiotensin I (1 μM) collected using a folded polyimide emitter, emphasizing the doubly protonated (*m/z* 649) and triply protonated (*m/z* 433) ion peaks. (b) MS/MS with CID performed on the triply protonated *m/z* 433 precursor ion. Several peaks have been assigned as *b*- and *y*-ion peptides (see inset).

tens-to-hundreds of such devices in a couple of hours, working in a conventional laboratory with no specialized resources (i.e., no cleanroom required). But an alternative method to supply the driving potential is to pattern a thin wire directly onto the substrate. An example of such an emitter is presented in Fig. 6(a); a 150-μm gold wire has been patterned on polyimide tape using conventional microfabrication techniques in a cleanroom facility. The procedure includes an important step: treatment of the surface of polyimide substrates with oxygen plasma. In initial experiments, we observed that when metal was deposited directly on the polyimide without pre-treatment, poor adhesion led to micro-cracks, resulting in a wire that was broken and not conductive. Thus, an oxygen plasma treatment was used to clean the surface and promote adhesion of the chromium seed layer to polyimide.[31]

After fabrication of a conductive wire, the substrate is folded into a cone, ensuring the wire runs directly to the emitter orifice. It was found that if patterned wires were greater than 500 μm wide, the applied voltage created a high electric field and caused undesirable corona discharge at the tip; 150-μm wire widths were selected because of the ease of fabrication and good electrical contact provided. A spectrum of Ultramark 1621 collected using an emitter with a microfabricated wire is shown in Fig. 6(b). The spectrum is comparable to spectra collected for the same analyte using both an unpatterned polyimide emitter and a pulled-glass capillary emitter (Fig. 3), demonstrating that the fabrication process does not have a negative effect on emitter performance. Although emitters with the integrated wire require additional preparation time, fabrication is fairly simple (consisting of a single photolithography step) and eliminates the need for an external wire to be interfaced to the system. In addition, this format suggests future possibilities for coupling the new emitters to digital microfluidics.[32]

CONCLUSIONS

We present a new emitter for nanoESI-MS, employing a piece of polyimide tape folded into a cone. In contrast to the conventional pulled-glass capillary emitters typically used for nanoESI-MS, the new emitters are simple to make and are much less expensive, costing only $0.07 USD 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 can be used for MS/MS analysis. In addition, the new emitters show similar performance and spray stability to pulled-glass emitters over extended periods of time. We acknowledge that the new folded emitters are not appropriate for all applications, as they are not compatible with in-line integration with chromatography; however, we propose these new emitters may be a welcome innovation for applications in which samples are analyzed by direct-infusion mass spectrometry (with no chromatography), such as evaluation of newborn blood samples for inborn metabolic disorders,[33] shotgun lipidomics,[34] and metabolomics.[35] Finally, we are particularly enthusiastic about the possibility of mating the new method to digital microfluidics (given the

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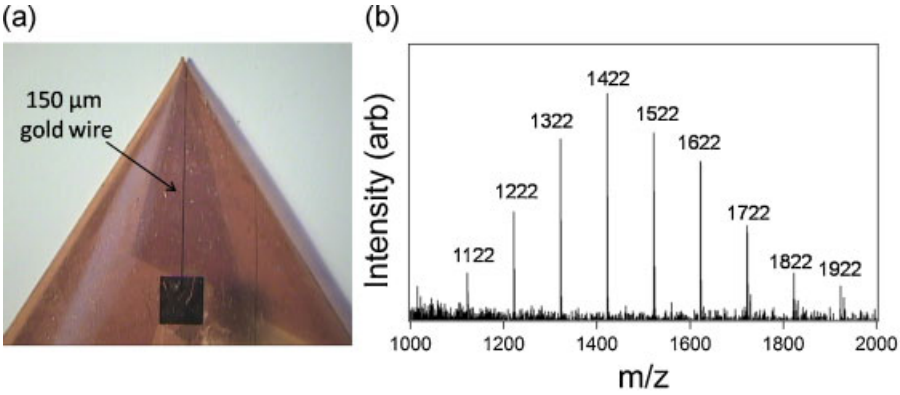

Figure 6. Folded polyimide emitter with a microfabricated wire for application of spray voltage. (a) Image of the emitter. The wire runs directly to the orifice to ensure contact with a solution placed in the tip. (b) Mass spectrum of Ultramark 1621 (1 μM) obtained using a folded emitter with microfabricated gold wire.

similarities in device substrates[36]), which could greatly expand the utility of this technique.

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REFERENCES

1. Yamashita M, Fenn JB. *J. Phys. Chem.* 1984; **80**: 4451.
2. Fenn JB. *Angew. Chem. Int. Ed.* 2003; **42**: 3871.
3. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. *Science* 1989; **246**: 64.
4. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. *Mass Spectrom. Rev.* 1990; **9**: 37.
5. Wilm MS, Mann M. *Int. J. Mass Spectrom. Ion Processes* 1994; **136**: 167.
6. Wilm M, Mann M. *Anal. Chem.* 1996; **68**: 1.
7. Karas M, Bahr U, Duluks T. *Fresen. J. Anal. Chem.* 2000; **366**: 669.
8. Juraschek R, Duluks T, Karas M. *J. Am. Soc. Mass Spectrom.* 1999; **10**: 300.
9. Schmidt A, Karas M, Duluks T. *J. Am. Soc. Mass Spectrom.* 2003; **14**: 492.
10. New Objective Online PicoTips®: SilicaTips™, © 2004 New Objective, Inc. Available: http://www.newobjective.com/products/tips_online_tips.html (accessed 13 July 2010).
11. Gibson GTT, Mugo SM, Oleschuk RD. *Mass Spectrom. Rev.* 2009; **28**: 918.
12. Le Gac S, Cren-Olive C, Rolando C. *J. Am. Soc. Mass Spectrom.* 2004; **15**: 409.
13. Freire SLS, Yang H, Wheeler AR. *Electrophoresis* 2008; **29**: 1836.
14. Muck A, Svatos A. *Rapid Commun. Mass Spectrom.* 2004; **18**: 1459.
15. Schilling M, Nigge W, Rudzinski A, Neyer A, Hergenroder R. *Lab Chip* 2004; **4**: 220.
16. Sikanen T, Tuomikoski S, Ketola R, Kostiainen R, Franssila S, Kotiaho T. *J. Mass Spectrom.* 2008; **43**: 726.
17. Mellors JS, Gorbounov V, Ramsey RS, Ramsey JM. *Anal. Chem.* 2008; **80**: 6881.
18. Hoffmann P, Hausig U, Schulze P, Belder D. *Angew. Chem. Int. Ed.* 2007; **46**: 4913.
19. Liuni P, Rob T, Wilson DJ. *Rapid Commun. Mass Spectrom.* 2010; **24**: 315.
20. Yin H, Killeen K, Brennen R, Sobek D, Werlich M, van de Goor T. *Anal. Chem.* 2005; **77**: 527.
21. Agilent Technologies 1260 Infinity HPLC-Chip/MS System, © Agilent 2000–2010. Available: http://www.chem.agilent.com/en-US/Products/Instruments/lc/1200serieshplc-chipmssystem/pages/gp38427.aspx (accessed 13 July 2010).
22. Ksoyonov S, Williams P. *Rapid Commun. Mass Spectrom.* 2001; **15**: 1890.
23. Wang HL, Liu J, Cooks RG, Ouyang Z. *Angew. Chem. Int. Ed.* 2010; **49**: 877.
24. Liu J, Wang H, Manicke NE, Lin J-M, Cooks RG, Ouyang Z. *Anal. Chem.* 2010; **82**: 2463.
25. Summary of Properties for Kapton® Polyimide Films, © DuPont, 2010. Available: http://www2.dupont.com/Kapton/en_US/assets/downloads/pdf/summaryofprop.pdf (accessed 5 September 2010).
26. Emmett MR, Caprioli RM. *J. Am. Soc. Mass Spectrom.* 1994; **5**: 605.
27. Moini M. *Rapid Commun. Mass Spectrom.* 1994; **8**: 711.
28. Straub RF, Voyksner RD. *J. Am. Soc. Mass Spectrom.* 1993; **4**: 578.
29. Shukla AK, Futrell JH. *J. Mass Spectrom.* 2000; **35**: 1069.
30. Han X, Aslanian A, Yates III JR. *Curr. Opin. Chem. Biol.* 2008; **12**: 483.
31. Cho SH, Kim SH, Lee JG, Lee N-E. *Microelectron. Eng.* 2005; **77**: 116.
32. Wheeler AR. *Science* 2008; **322**: 539.
33. Rashed MS. *J. Chromatogr. B* 2001; **758**: 27.
34. Stahlman M, Ejsing CS, Tarasov K, Perman J, Boren J, Ekroos K. *J. Chromatogr. B* 2009; **877**: 2664.
35. Erve JCL, Beyer CE, Manzino L, Talaat RE. *Rapid Commun. Mass Spectrom.* 2009; **23**: 4003.
36. Abdelgawad M, Freire SLS, Yang H, Wheeler AR. *Lab Chip* 2008; **8**: 672.

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