NANOELECTROSPRAY EMITTERS: TRENDS AND PERSPECTIVE

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The benefits of electrospray ionization are many, including sensitivity, robustness, simplicity and the ability to couple continuous flow methods with mass spectrometry. The technique has seen further improvement by lowering flow rates to the nanoelectrospray regime (<1,000 nL/min), where sample consumption is minimized and sensitivity increases. The move to nanoelectrospray has required a shift in the design of the electrospray source which has mostly involved the emitter itself. The emitter has seen an evolution in architecture as the shape and geometry of the device have proved pivotal in the formation of sufficiently small droplets for sensitive MS detection at these flow rates. There is a clear movement toward the development of emitters that produce multiple Taylor cones. Such multielectrospray emitters have been shown to provide enhanced sensitivity and sample utilization. This article reviews the development of nanoelectrospray emitters, including factors such as geometry and the manner of applying voltage. Designs for emitters that take advantage of multielectrospray are emphasized. © 2009 Wiley Periodicals, Inc., Mass Spec Rev 28:918–936, 2009

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I. INTRODUCTION

Electrospray is a well-known phenomenon born from subjecting the meniscus of a liquid emitted from an orifice to a high voltage applied between the liquid and a counter electrode, elongating the liquid to a cone-jet, leading to the generation of nanometer-sized monodisperse droplets. The pioneering study of electrospray was carried out by the physicist John Zeleny in 1914 (Zeleny, 1917) and theoretical descriptive models were explained by Taylor (1964). The wide applicability of this technique has been realized in various fields such as thin film deposition, nanoparticle and nanofiber synthesis, the development of thrusters for microsatellite positioning and electrospray ionization mass spectrometry (ESI-MS) (Salata, 2005; Si, Byun, & Lee, 2007). The applicability of electrospray to the ionization of biomolecules was initially described by Dole et al. (1968) and later convincingly fine-tuned and widely popularized by Fenn in the 1980s. For this ground-breaking contribution, Fenn shared the 2002 Nobel prize with Tanaka who worked on the development of Matrix-Assisted Laser Desorption Ionization (MALDI), the soft ionization method and cousin to ESI (Fenn et al., 1989; Tanaka, 2003).

The last decade has seen the unprecedented growth of ESI-MS to become an indispensible analytical tool that finds utility in various disciplines including proteomics, metabolomics, glycomics, and as a compound identification tool for synthetic chemists. The appeal for its use is largely due to its unparalleled ability to analyze large molecules without fragmentation, its characteristic high sensitivity, high versatility and wide applicability, and its ability to couple directly to liquid chromatography (LC) and capillary electrophoresis (CE) (Cech & Enke, 2001). The popularization of ESI-MS can also be partially attributed to the progression of research attention to the analysis of the large numbers of gene-expressed proteins (proteomics), which became popular after the completion of the human genome project in 2003. Proteomics has taken center stage because of its promise for the discovery of disease biomarkers and novel therapeutic targets, especially for cancer and cardiovascular diseases (Kubicek et al., 2004; Smith, Shen, & Tang, 2004). The concomitant development of more powerful mass analyzers, some with very high duty cycles such as Fourier transform—ion cyclotron resonance (FT-ICR), triple-quadrupole (QqQ), and quadrupole/time-of-flight (QTOF), that can be interfaced to ESI, has also largely contributed to the development of the field in general.

The electrospray is fundamentally achieved by spraying a solution of analyte through an emitter across a potential difference (Kebarle & Tang, 1993). Ions present in solution and those formed by electrochemical reactions with the solvent at the electrode migrate toward the counter electrode (the MS orifice plate), causing the formation of the Taylor cone as the ions bring solvent with them. At the tip of the cone, where the electric field is very high, the cone becomes unstable and a liquid jet is emitted. The jet becomes unstable a short distance after and becomes a set of charged droplets. The droplets have an excess of positive ions at the surface (in positive ion mode) and, as the droplets get smaller due to solvent evaporation, they reach a limit where they undergo fission in an uneven manner to give several much smaller ion-enriched droplets. Those droplets are subjected to further desolvation and fission events until they are small enough to produce gas-phase ions.

There are two possible mechanisms for the production of gas-phase ions. The first, assumed to be the case in the early studies by Dole et al. (1968), involves continued desolvation and droplet fission until there is only a single ion in a droplet, whereby further desolvation releases the gas-phase ion. The second possibility, proposed by Thomson and Iribarne (1979), is that small highly charged droplets begin to emit gas-phase ions rather than continue Coulombic fission. In either case, the gas-phase

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ions enter the mass spectrometer where they are analyzed and detected.

While the emitter is a small component of the overall ESI process, the success of electrospray is critically determined by the emitter's geometry as well as the flow rate it allows. The development of emitters has thus seen a revolution in an effort to improve the electrospray process. In Dole's first demonstration of the production of a ''molecular beam of macroions,'' a stainless steel hypodermic needle with an outer diameter of approximately 200 μm was used (Dole et al., 1968). In Fenn's experiments (Fenn et al., 1989) a similar needle was employed with an outer diameter of 100 μm. However, the efficiency of ionization generated by such large diameter emitters is poorer due to the large charged droplets that emanate from them, requiring a longer series of Coulombic explosions prior to the formation of gas phase ions. With the growing need for higher sensitivity, which is directly related to the efficiency of ionization, smaller aperture emitters (∼50 μm) were developed for what was termed ''microelectrospray'' (Emmett & Caprioli, 1994). This was found to improve ionization efficiency, especially with the tandem use of coaxial nebulizing gas with milliliters per minute flow rates, otherwise referred to as ''ionspray'' or ''pneumatically assisted electrospray.'' Some of the gene-expressed proteins of key interest in the proteomics community often occur at trace levels, necessitating extremely sensitive detection methods.

Wilm and Mann (1994) described theoretical derivations showing a strong correlation between liquid flow rate and the size of the droplets emanating from the Taylor cone (assuming a constant cone angle of 49.3°). At nanoliters per minute flow rates the droplets were small enough (reported to be ∼180 nm) to be capable of yielding gaseous ions without requiring as many fission events or as much desolvation. This was confirmed experimentally and led to the birth of nanoelectrospray (flow rates <1,000 nL/min) ionization, a key revolution from conventional ESI (flow rates >1 μL/min). There is some controversy over terminology; ''nanoelectrospray'' is traditionally restricted to flow rates less than 100 nL/min and ''microelectrospray'' is 100–1,000 nL/min, but for the purposes of this review nano-electrospray will cover sheathless interfaces using flow rates less than 1,000 nL/min.

The principal advantages of nanoelectrospray include improved ionization efficiency and ion transmission primarily attributed to the emanation of small droplets, which affords increased surface charge per analyte molecule and results in increased sensitivity. A recent study demonstrated the improved efficiency of nanoelectrospray ionization MS, as defined by the number of ions leaving the emitter that are detected by the MS, where lowering the flow rate from 1,000 to 4 nL/min caused an essentially linear increase in efficiency, which was typically about 1% at its maximum (El-Faramawy, Siu, & Thomson, 2005). This study also showed a strong variation from emitter to emitter, further demonstrating the importance of the emitter in the success of the process. The low flow rates used also mean better sample economy, and indeed less than 1 μL and only femtomoles of analyte can be used for tandem MS investigations when flowing at 25 nL/min (Wilm & Mann, 1994, 1996). Moreover, improved desolvation at such low flow rates alleviates the need for nebulizing gas.

Nanoelectrospray has also been found to greatly minimize ion suppression and matrix effects, which can seriously hinder conventional ESI. Schmidt, Karas, and Dulcks (2003) studied ion suppression effects as an indicator of nanoelectrospray behavior, finding that highly surface-active ions strongly suppress more hydrophilic ions at higher flow rates, owing to the enrichment of surface ions in uneven droplet fission. At lower flow rates (<50 nL/min), however, ion suppression was nearly eliminated, presumably because the charged droplets are sufficiently small that a higher number of all analytes are converted to gas-phase ions and detected. With these key advantages nanoelectrospray will likely be the technique of choice for proteomics and other areas of large-scale biomolecule analysis such as metabolomics and glycomics (Want et al., 2007).

The development of nanoelectrospray emitters has been advanced by many groups. The interest in emitter development is mainly because of the pivotal role they play in ensuring the success of nanoelectrospray. Indeed, the size, shape, reproducibility and stability of the Taylor cone are highly dependent on the emitter tip architecture. However, the comparison of emitter performance between studies becomes very difficult as it is ultimately dependent on a number of variables besides tip geometry. The field of nanoelectrospray has yet to adopt a ''standard'' method of testing to enable the direct comparison of emitter performance in different studies. Perhaps future studies can utilize a similar set of experiments to probe emitter performance. Below, we suggest a set of conditions/experiments that would assist nanoelectrospray practitioners in assessing the usefulness of novel emitter designs.

Sensitivity and stability of an emitter design has typically been probed using spray or ion current (total or extracted) generated by the constant infusion of a sample. We suggest that ion current is a good monitor of both sensitivity and stability as it measures ionization efficiency rather than the generation of charged droplets. It follows that sensitivity could be quoted as a detection limit at a particular signal/noise ratio and stability described as the relative standard deviation of either the total or extracted ion current.

An often-overlooked aspect of electrospray experiments is the voltage applied to generate the spray. While it is true that increasing voltage may raise the spray current or total ion current, this is because the analyte ion is overwhelmed by other ions generated in electrochemical processes and is therefore not meaningful. At some point the Taylor cone breaks down and the results become irrelevant. If the voltage is too low, on the other hand, the liquid leaving the emitter builds up in favor of forming a Taylor cone, and no ion current is measured. In fact, research suggests that the cone-jet mode of electrospray occurs only at a relatively narrow range of applied potential, the value of which is further dependent on solvent composition, flow rate and emitter position (Alexander, Paine, & Stark, 2006; Marginean et al., 2007, 2008; Paine, Alexander, & Stark, 2007). Care should be taken to characterize the range of applied voltage and report it under a given set of conditions.

Furthermore, ionization efficiency is very much dependent on the nature of the analyte chosen so that a direct comparison between studies that utilize different analytes is difficult. We propose three possible analytes that could be used as comparison samples. These choices are predicated on their use in the

literature, their sensitivities in electrospray experiments, and the fact that they span a relatively wide mass range. These include: dodecyltrimethyl ammonium (DDTMA) bromide (*m*/*z* 228), which has a high surface activity and provides high sensitivity/efficiency; leucine enkephalin, a small, reasonably hydrophobic peptide that shows good sensitivity and generates a single ion at *m*/*z* 556; and apo-myoglobin (17 kDa) as this has become a commonly used protein analyte for ESI characterization.

It follows that the emitter should be tested at a number of different flow rates that span the nanoelectrospray regime. We suggest using flow rates from 500 to 10 nL/min as this range includes flow rates associated with nano-LC-MS as well as flow rates more traditionally associated with nano-ESI. The generation of electrospray is a complex interplay of solution composition and its associated surface tension, applied voltage, and emitter position and morphology. We suggest characterizing the emitter performance using a solvent composition that ranges from highly aqueous to highly organic (e.g., MeOH or acetonitrile) and that the practitioner takes care to ensure that the emitter position is optimal. In this way emitters could be compared at either end of a typical LC solvent gradient. It is also important for the comparison of emitter performance that all these parameters are reported.

Finally, since emitter robustness (as determined by the deterioration of electrospray performance, usually caused by clogging or loss of conductivity) is a well-known issue, the emitter should be used and monitored for extended periods (e.g., hours or days), and perhaps a sample known to easily clog emitters such as Hanks' salt solution should be used to further probe resistance to clogging.

The first section of this review will chronicle the development of these single aperture emitters with varied geometries and modifications. Many of these emitters, however, have some serious limitations including a susceptibility to clogging due to a constricted aperture, a limited range of possible flow rates, and poor reproducibility in manufacture, impeding online methods and the much needed reproducibility for quantitation.

To address these and other limitations associated with single aperture tapered tips, a new paradigm has emerged taking advantage of multiple spray. The use of multinozzled tips has been found to significantly improve sensitivity and to extend the lifespan of emitter tips by reducing clogging tendencies. The increase in sensitivity associated with multi-ESI emitters has been modeled by Tang et al. (2001), derived from the original relationship between spray current and flow rate by Fernandez de la Mora and Loscertales (1994), where total spray current is shown to be proportional to the square root of the number of emitters, assuming each emitter operates in cone-jet mode (see Eq. 1). As such, a greater number of electrospray emitters increases the spray current relative to a single emitter at the same total flow rate. Note, however, that an increase in spray current does not necessarily translate into a proportional increase in a given ion signal, but that relationship is far more complicated.

In this work we refer to the multiple spray concept as "multispray," although multispray or "multijet electrospray" has been observed before using single emitters (Ikonomou, Blades, & Kebarle, 1990). Those reports represent extreme cases of high voltage whereby multiple Taylor cones can be seen emanating from various points around the rim of a single aperture, but clearly any sensitivity increases arising from multispray do not apply in such cases and so they will not be discussed here.

Interestingly, in addition to ESI-MS, several examples of multiple electrospray devices in the literature describe other applications utilizing aerosol science, such as for microthrusters and deposition of thin films (Almekinders & Jones, 1999; Salata, 2005; Si, Byun, & Lee, 2007). The multielectrospray concept, however, is starting to find great utility in ESI-MS and we predict future developments will largely involve the development of such platforms. In the second portion of this review we shall discuss the different approaches that have been developed for multi-ESI emitters, and try to envision a direction that this multispray technology will take. Most of the multiplexed ESI devices were mainly designed in the microchip format, and for such microfluidic devices coupled with ESI-MS there are some excellent and thorough reviews that have been published (Oleschuk & Harrison, 2000; Sung, Makamba, & Chen, 2005; Lazar, Grym, & Foret, 2006; Koster & Verpoorte, 2007). While we shall discuss some of the multi-ESI-MS microchip-based platforms already included in the above reviews for completeness, our emphasis in this review is on novel non-chip based multi-ESI emitters developed in recent years.

$$I_{\text{total}} = \sqrt{n} I_{\text{s}} \tag{1}$$

where I_{total} is the total multielectrospray spray current, n the number of emitters, and I_{s} the spray current of an individual emitter.

II. CLASSIFICATION AND APPLICATIONS OF NANOELECTROSPRAY EMITTERS

A. Single Electrospray Emitters

The design of emitters for nanoelectrospray has two primary concerns, namely the geometry of the tip and the way potential is applied for the electrospray process. The former is concerned with the formation of a stable cone jet at the tip exit at low flow rates while maintaining robustness and practical working conditions. This section focuses on the development of individual emitters, including their tip geometry, other surface modifications that improve spraying, and the method of electrical contact, as well as nanoelectrospray emitters integrated with other functions for a variety of applications.

1. Emitter Geometry

The establishment of nanoelectrospray at true nano-flow rates by Wilm and Mann in the mid 1990s (Wilm & Mann, 1994, 1996) introduced a simple design for a needle-like electrode capable of producing a stable Taylor cone at flow rates down to 20 nL/min, a design that has remained essentially unchanged in many practical applications to date. In its basic form this emitter is a glass tube that is heated to melting and drawn until separated, whereby both the outer diameter (o.d.) and inner diameter (i.d.) dimensions are tapered to a very fine point. With the proper equipment and technique, such tubes can be pulled fairly reproducibly to give

tips with uniform dimensions. The tip opening at this stage, however, is still blocked or too small to allow flow. In initial experiments the tip was opened by touching it against the interface plate and, by a combination of physical and electrical stresses, the i.d. at the exit became 1–2 μm. Clearly this approach provides too much uncertainty, especially since the tip i.d. was subsequently found to be of critical importance to the electrospray process. Instead, the tip is usually etched with a hydrofluoric acid (HF) solution to provide better control over the dimensions of the opening. Emitters of this type are commercially available from companies such as New Objective, Inc., Woburn, MA, USA, and World Precision Instruments, Sarasota, FL, USA and are used for offline analysis of small volumes of sample without the use of a pump.

What became more widely used was the much thinner fused silica capillary tubing. Such tubing was already available for a variety of applications, was generally more robust, and allowed online coupling with the small-scale separation techniques that were being used in conjunction with ESI-MS. The use of smaller i.d. capillary (i.e., <20 μm) was complicated by the excessive back pressure it induced, since even short lengths can exceed the capabilities of many pumping systems, but the ability to more easily produce tapered tips with very small i.d. (<5 μm) may overcome this drawback. To this end Valaskovic et al. (1995) developed a pulled fused silica capillary emitter, the tip of which was opened using HF etching. This emitter design, shown in Figure 1, is currently the most widely used in nano-ESI applications.

The trend in tip exit dimensions went to ever smaller inner diameters, since Wilm and Mann's initial theory and experiments suggested that smaller i.d.'s were essential for allowing very low flow rates which were in turn necessary for optimizing the desorption and ionization efficiency of the sample by decreasing

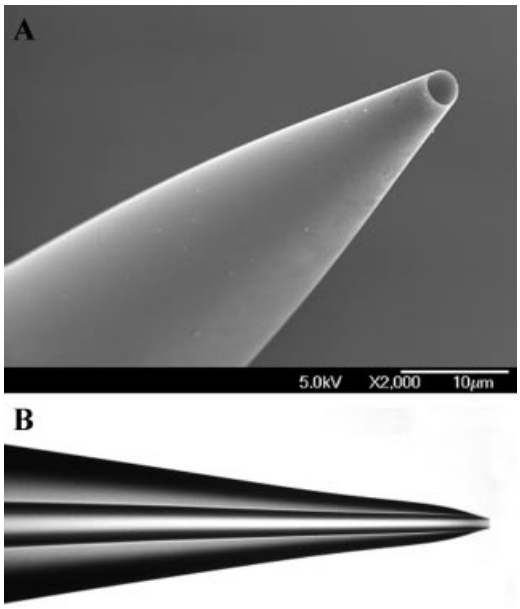

FIGURE 1. SEM image (**A**) and optical image (**B**) of a typical drawn capillary emitter where both inner and outer diameters are tapered. Images courtesy of New Objective, Inc.

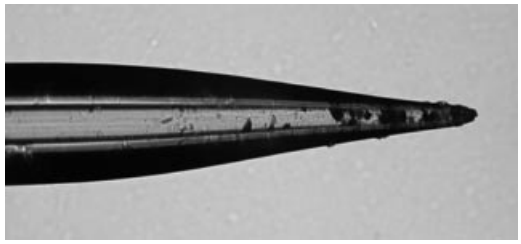

FIGURE 2. Optical image of a tapered emitter that has clogged with normal use.

droplet size. While determining the optimal flow rate depends on other factors such as outer diameter, channel taper and solvent considerations, the inner diameter is the most effective and easiest to vary. All known studies relating tip i.d. to performance suggest the optimal flow rate for generating the smallest droplets decreases with decreasing diameter, for both tapered (Xiong et al., 2007) and non-tapered (Covey & Pinto, 2002) emitters. Interestingly, it was also noted that the smallest i.d. capillaries (thus providing the smallest droplets) allowed the detection of ions that more closely resembled those present in the solution phase, presumably due to fewer fission events (Karas, Bahr, & Dulcks, 2000; Zampronio et al., 2004).

A disturbing aspect of the trend toward smaller i.d. capillaries is the inherent tendency for small openings to become clogged, such as that shown in Figure 2, a problem exacerbated by the tapered channel through which the solution must pass. Even scrupulously filtered solvents contain or pick up particulates that can eventually build at the emitter aperture and lead to clogging. Davis et al. (1995) inserted a polyvinylidene fluoride membrane filter in their drawn glass emitter to improve upon Wilm and Mann's original design to prevent such clogging, but even with filtering their emitters lasted only 8–12 hr on average before clogging. For Xiong et al. (2007) routine studies were limited to ≥ 10 μm i.d. tapered fused silica capillary tips since smaller i.d.'s clogged too rapidly.

In an effort to reduce clogging and improve fabrication methods, several research groups were etching fused silica capillaries to provide a tapered architecture at the tip. In this case a small section of the polyimide coating of the capillary is removed and the end is immersed in an etchant such as hydrogen fluoride (e.g., 49% aq. HF). The etching process here is isotropic, effectively reducing the outer diameter for a short length of the capillary to provide the tapered tip. The first experiments using this technique (Wahl et al., 1992) showed that the inner diameter was slightly widened and the capillary wall at the exit was very thin, depending on etching time. Later (Emmett & Caprioli, 1994) water was flowed through the capillary to prevent etching of the inside of the capillary and thus maintaining constant i.d. More recently (Kelly et al., 2006) Smith improved upon the reproducibility of the procedure by allowing the etching to continue until the tip was completely etched, at which time the liquid/air barrier drastically slowed further etching and all capillaries were thus etched to the same extent. Figure 3 shows a capillary part-way through this etching process alongside another that is completely etched. An important feature of these etched capillaries is that the inner diameter is not tapered, providing

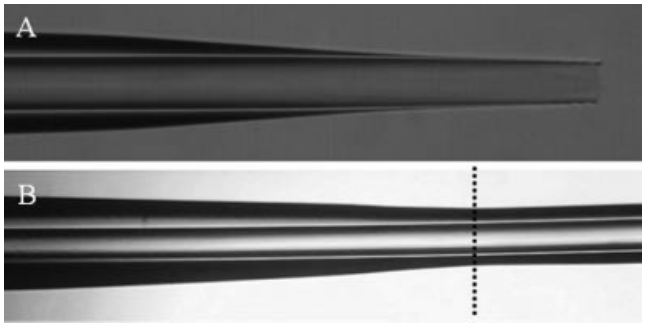

FIGURE 3. Optical images of a capillary emitter that is tapered in the outer diameter only by (**A**) complete chemical etching and (**B**) partial chemical etching (showing where the fluid level would be). Reproduced with permission from Kelly et al. (2006). Copyright 2006 American Chemical Society.

some relief from the clogging problems of pulled tips, but that the o.d. is tapered and the wall is thin which should aid the stabilization of the Taylor cone. Another effort to taper only the o.d. involved the physical grinding of the tip with sandpaper, although the capillary wall at the exit was still quite thick (Kriger, Cook, & Ramsey, 1995).

2. *Other Tip Modifications*

It has been generally accepted that a tapered design is the most effective way to maintain a stable Taylor cone at the emitter exit. However, the important feature of these emitters is that the droplet/Taylor cone is supported by the i.d. of the exit, the smallest possible orifice. Ultimately the issue comes down to a matter of solvent wetting. In the case of tapered emitters the liquid leaving the emitter must wet the outside of the needle which is situated at a large angle to the exit. For unmodified capillaries the tip face is situated at 90° to the exit and is thus much more easily wetted by the solvent, especially for silica in combination with solvents containing significant amounts of water (which is very common). In these cases, the solvent wets the tip face and the Taylor cone is much larger, sometimes with a base supported by the emitter's entire diameter. In fact, Taylor cones typically form at the point of the emitter closest to the MS counter-electrode and, as a consequence of wetting various parts of the tip, Taylor cones can be observed emanating from various parts of the surface at different times (Wahl et al., 1992).

Aside from tapering the end, another approach to prevent wetting of the tip face is to chemically modify it to make the face unwettable by the solvent. Since most applications use more aqueous based solvents, modification of the tip face to make the entire end hydrophobic would be the most obvious strategy. In this case the solvent would not wet the tip face and the Taylor cone should be primarily supported by the inner diameter of the capillary. To this end, hydrophobic coatings such as polymers or a trimethylsilyl monolayer (manuscript in preparation) show much improved sensitivity and Taylor cone stability. With such modified emitters the reduced size of the Taylor cone can be observed, which makes controllable cone-jet nanoelectrospray possible and gives better mass spectra. In another example, Liu et al. (2004) used a perfluoroalkyl coating to restrict the base of their Taylor cone to the large (75 μm) i.d. capillary exit. In this case, the Taylor cone was further stabilized by the presence of a carbon fiber protruding from the end of the open capillary that provided a point from which the Taylor cone could emanate (Liu et al., 2004; Sen et al., 2006).

Even for tapered emitters there is a certain wall thickness at the emitter exit that is wetted by the solvent during electrospray, and these emitters can also benefit from a hydrophobic coating. Tojo (2004) developed an etched emitter coated with a fluorinated polymer, commercialized by Omniseparo-TJ, Amagasaki, Japan, as FortisTip™, which is claimed to provide stable electrospray for highly aqueous samples. In another example the commercially available NiagaraFlow emitters (Nanogenesys, Ithaca, NY, USA) were coated with a hydrophobic acrylic paint to provide better sensitivities (Choi & Wood, 2007).

Using a different approach, Su, Marecak, and Oleschuk (2008) altered the wetting characteristics of the tip face by roughening it with sandpaper. The roughened end, analyzed by AFM and found to have surface features on the order of ~5–15 μm, was proposed to be involved in a Wenzel contact state with the liquid in which the droplet penetrates the spaces between the surface features. Under these conditions the Taylor cone was much more stable and, even though it was supported by the entire o.d., the contact angle was much smaller due to the altered surface energy. Advantages of using such large-bore emitters are of course the resistance to clogging and lack of back pressure but, as for any process dependent on wetting behavior, these emitters are dependent on solvent composition.

3. *Mode of Electrical Contact*

Electrospray requires a potential difference to be applied to the emitter, with the MS inlet as the counter-electrode. Typical emitters, however, are made of non-conductive silicate materials and as such making electrical contact with the emitter has been a challenge. Several modes of electrical contact have been used successfully, developed in parallel, including the insertion of a wire into the emitter, incorporating an electrode into the system upstream of the emitter making a liquid junction, and coating the outside of the emitter with a conductive material (Fig. 4).

The simplest emitters are not themselves conductive, so a universal mode of electrical contact that does not rely on the emitter is the most convenient. There are several examples using a wire inserted into the capillary to establish an electrochemical junction with the liquid. Early microspray attempts involved inserting a small gold wire into the emitting end of a CE/MS interface (Fang et al., 1994), or a platinum wire through a small hole drilled into the emitter near the exit (Cao & Moini, 1997). A similar approach (Wahl & Smith, 1994) had this drilled hole filled with a conductive epoxy. A later nanospray example (Fong & Chan, 1999) featured a gold-plated tungsten wire, mounted on a screw for positional control, inserted from the back of a pulled borosilicate glass capillary.

In a more recent study using nanoelectrospray in the determination of association constants for non-covalent complexes (Zampronio et al., 2004), platinum, gold, and tungsten

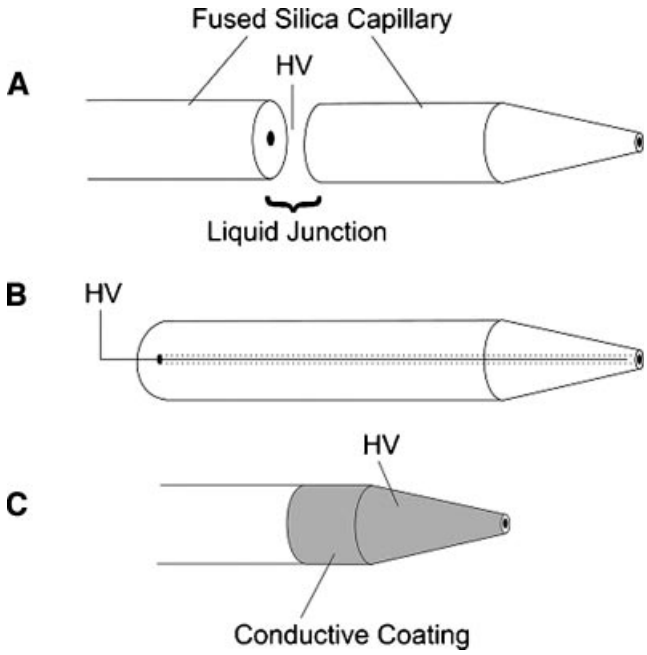

FIGURE 4. Schematic of various electrical contact modes; (**A**) liquid junction; (**B**) wire placed at the emitter exit; (**C**) conductive coating.

wires were compared. Visual analysis of the mass spectra obtained, combined with calculated association constants, led the authors to conclude that no significant differences existed between the three types although continuous operation did not last longer than 15 min. Longer times (>90 min) were observed to cause significant drops in pH for unbuffered solutions due to oxidation reactions at the platinum or stainless steel electrode in the emitter tip (Van Berkel, Asano, & Schnier, 2001). The stainless steel, iron and copper wires they tested, on the other hand, showed significant signs of anodic corrosion within 25 min, together with the presence of metal ion complexes in the mass spectra. Care must therefore be taken in the choice of wire for this kind of set-up to avoid undesired electrochemical processes. Furthermore, wire insertion becomes complicated as the tubing i.d. gets smaller, and is not suitable at all in cases where the tip contains some other solid, such as when it is packed with a stationary phase.

The simplest and most convenient method of applying voltage is a liquid junction provided by an electrode in contact with the solution upstream of the emitter, usually in the form of a union or tee. Again, this method is useful for any emitter type or design including those with structures held within them. The convenience of this approach has led to its proliferation for electrospray. Initially such an approach was used for conventional electrospray by applying a voltage to the sheath liquid that served as the spray potential (Lee et al., 1989). In later nanospray experiments the voltage was applied directly to the analyte solution *via* a metal holder (Emmett & Caprioli, 1994). To demonstrate the versatility of this approach stable nanoelectrospray was established even when the liquid junction was placed upstream of a 100 μm i.d. (pulled to 2 μm at the exit), 10 cm-long packed column that also served as an emitter (Gatlin et al., 1998). One potential drawback of this approach is that the voltage applied may be higher or more variable than if electrical contact is made at the tip exit. Jackson and Enke (1999) studied the relationship between applied voltage and current for either metal emitters or pulled glass emitters with an upstream liquid junction. It was determined that the electrical resistance offered by the solution, though relatively small compared to the air gap, was significant and would increase the voltage required to produce the same current. Since the electrochemical contact with the liquid occurs at the point of the electrode closest to the counter-electrode (Enke, 1997), this means that contact points near the emitter exit, such as for metal emitters, metal-coated emitters or emitters with inserted wires near the exit, will not possess significant solution resistance while liquid junctions far removed from the exit will.

Several other examples exist where liquid junctions are used outside of the capillary. One such example involves an open hole where a CE capillary is connected to the nanoelectrospray emitter, where the entire union is immersed in electrolyte in a pressurized vessel containing the electrode (Fanali et al., 2006). Another example uses microdialysis tubing as a union between the CE capillary and emitter which is immersed in liquid inside a plastic pipette tip also containing an electrode (Severs, Harms, & Smith, 1996). Edwards et al. (2006) actually etched a porous hole in the side of their CE capillary upstream of the emitting end that served as a junction, this section being immersed in electrolyte in the presence of an electrode.

When the emitter itself is conductive, the voltage can be applied directly to the emitter. The obvious type is an emitter fashioned from metal such as stainless steel. However, the fabrication of small i.d. emitters in this medium is difficult and hence these emitters are not widely available for nanoelectrospray. While a stainless steel emitter would have the benefit of much improved robustness and resistance to heat (Yamada, Suzuki, & Hirayama, 2006), electrochemical instability has been a problem in microspray applications. In a recent article (Chen & Cook, 2007) all the stainless steel emitters studied showed susceptibility to electrical discharge causing corrosion that adversely affected experiments, a problem that worsened as the emitter aged.

The simplest design for a conductive emitter is to render a traditional silicate emitter conductive by coating it with conductive material. Only contact with the solution is required, and in this case it would be briefly at the exit of the emitter. This suggests that only the exit needs to be coated, but generally more of the emitter is coated to allow for the application of voltage, typically by a clip or wire with the assistance of conductive paint or adhesive. This approach has been used extensively for some time, and was the method of choice in the beginning of nanoelectrospray. Wilm and Mann's early experiments (Wilm & Mann, 1994, 1996) involved the vacuum deposition of gold onto the distal end of the emitter, with voltage applied directly to the external surface of the tip. Valaskovic et al. (1995), Valaskovic, Kelleher, and McLafferty (1996), and Valaskovic and McLafferty (1996a) also used this technique, and their initial success led to the founding of New Objective, Inc. Such emitters, however, had short lifetimes on the order of 15 min to 3 hr, since the thin deposited layer is susceptible to deterioration to an extent capable of altering the required voltage or positioning for stable electrospray. In some cases the gold layer was observed to have

flaked off or peeled back. To improve the robustness of Au-coated emitters, Valaskovic and McLafferty (1996b) overcoated the gold layer with a 10–50 nm thick layer of insulating SiO/SiO_2 by thermal evaporation and deposition. This layer improved lifetimes to 1–2 hr on average, giving stable spray even with the occurrence of arcing. This general approach is still used commercially by New Objective, Inc. to produce Au-coated emitters. Attempts were made by other groups to improve the robustness of gold-coated emitters by including an adhesion layer undercoating. Kriger, Cook, and Ramsey (1995) utilized (3-mercaptopropyl)trimethoxysilane, a bifunctional reagent that condenses onto the silica surface of the emitter leaving a thiol moiety exposed, to better adhere the gold taking advantage of its natural affinity for the soft ligand. In another example (Barnidge et al., 1999) a chromium layer was first deposited onto the emitter's surface using an electron beam to provide a metallized layer that better adhered to the silica prior to the vacuum deposition of the gold layer. A vacuum-deposited gold layer was itself used as an undercoating of sorts for Thibault and co-workers (Bateman, White, & Thibault, 1997; Kelly, Ramaley, & Thibault, 1997), who augmented their gold layer by electroplating a much thicker gold layer.

Nilsson and Markides at Uppsala University have developed a number of nanoelectrospray tips based on conductive particles embedded within polymer. The first of these emitters was coined ''Fairy Dust'' for its 2 μm gold particles sprinkled onto a polyimide coating, shown in Figure 5 (Barnidge, Nilsson, & Markides, 1999). Such an arrangement was found to work well without further treatment to give stable electrospray for remarkably long periods (several weeks), although mass spectra were not taken during this time. Using a similar technique, they went on to embed graphite powder in a coated polyimide layer to make the tip conductive, calling it ''Black Dust'' (Nilsson et al., 2001). This emitter was as stable as the Fairy Dust emitter, but was much more cost-effective than using gold. As an extension to this approach a polypropylene/graphite powder mixture was used as a conductive coating and, more importantly, as the emitter material itself (Wetterhall et al., 2002). Known as ''Black Jack,'' such emitters were found to spray well over the long term with increased flexibility and resistance to electrical discharge. Graphite alone was used as a conductive coating for conventional pulled tips applied as a colloidal dispersion (Zhu et al., 2002) or by vacuum deposition (Smith & Wood, 2003) in other applications to achieve stable nanospray for several hours of continuous operation.

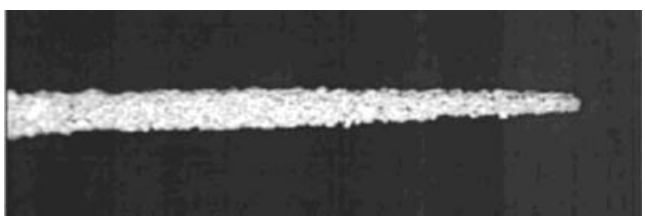

FIGURE 5. Optical image of a tapered emitter coated with polyimide and sprinkled with gold dust. Reproduced with permission from Barnidge, Nilsson, and Markides (1999). Copyright 1999 American Chemical Society.

A different kind of carbon electrode was used by Liu and Knapp in the form of a pointed carbon fiber (Liu et al., 2004; Sen et al., 2006). Experimental and modeling studies showed that a carbon fiber, etched to a point, could be affixed to the inside of a capillary emitter and protrude ~20–30 μm from the tip to stabilize a small Taylor cone. The entire emitter assembly was coated with gold by vacuum deposition, giving electrical contact to the pointed tip. According to the authors, this approach imparts versatility to the spraying conditions, and stable spray was observed up to 15–20 straight hours, largely limited by the gold/graphite connection being damaged by arcing.

As an alternative to metallic coatings, conductive organic polymers have also been used to coat silicate-based emitters. A number of publications have emerged from White and co-workers (Maziarz et al., 2000; Bigwarfe, White, & Wood, 2002; White & Wood, 2003) describing the production of pulled tips coated with polyaniline that have been commercialized by Nanogenesys as ''NiagaraFlow.'' The polymer adheres to silica better than deposited gold and is much more resistant to electrical discharge, making it more robust than conventional emitters and extending tip lifetime by several hours.

In a comparison of modes of electrical contact, Wetterhall et al. (2003) used chronoamperometry and cyclic voltametry to interrogate the electrochemical stability of pulled glass tips coated with noble metals (and various types of adhesion layers), polyaniline, and their own ''Black Dust'' and ''Black Jack'' formulations for graphite-based tips. With these techniques shorter lifetimes are expected as they involve electrochemical activity across the entire coating, not just at the tip. In their investigation, they found that the polyaniline-coated tips did not give an electrochemical response and were not tested further. The metal-coated tips showed significant deterioration and loss of coating, in contrast to their graphite-embedded polymer coating. The latter, however, was shown to be passivated, although it still gave stable spray. Another comparative study was done by Smith et al. (2006) for the analytical performance of tips coated with Au/Pt and their own graphite-coated and polyaniline-coated emitters. In their investigation they concluded that the choice of emitter depends on the application. Metal-coated emitters gave the strongest signal in positive ion mode, but suffered from degradation due to discharge especially in negative ion mode. They went on to suggest that other performance indicators such as signal linearity are similar for all emitters, and that there may be a difference in spray stability depending on the mass spectrometer. In a study of oligonucleotides in negative ion mode (Xiong et al., 2007) the performance of a gold-coated emitter was compared with that of a stainless steel emitter and an uncoated emitter (using a liquid junction) with similar tip geometries. They concluded, based on recording the same mass spectrum 10 times for each emitter type under the same spray conditions (no attempt was made to extend spraying time), that the distal metal-coated emitter was the best for them. This emitter gave 5-fold stronger signal for the analyte of interest than the metal emitter and 10-fold stronger than the uncoated emitter. Perhaps of more importance for them was the presence of metal ion adducts in their spectra when using the stainless steel emitter or stainless steel liquid junction. Zampronio et al. (2004) found no significant differences in the determination of association constants of non-covalent complexes between gold- or

platinum-coated tips and uncoated tips (using an inserted wire for contact), although their experiments were decidedly short ($\leq$15 min).

4. Integrated Emitters

The potential for nanoelectrospray emitters, with their small exit dimensions, to be packed with solid material was realized very early on. In fact, Wilm and Mann's (1996) and Emmett and Caprioli's (1994) initial reports included proof-of-principle experiments utilizing short beds of reversed phase packings for pre-concentration and desalting. Microscale liquid chromatography was first accomplished within a nanoelectrospray emitter around the same time using packed particles (Davis & Lee, 1998; Gatlin et al., 1998) and porous polymer (Moore et al., 1998) for the reversed phase separation of peptides for protein identification. Even with a very thin wall at a 2 μm i.d. tip, it was found that the emitter would hold at least 8,000 p.s.i. (550 bar) during packing (Gatlin et al., 1998). In spite of this apparent durability, emitters have been produced containing frits within the tip for packing purposes and are commercially available. An example of such a tip is shown in Figure 6.

The utility of packed emitters is clear, mostly inherent in the greatly reduced dead volume for online techniques compared to coupling with an emitter designed only for spraying. The use of emitters for this purpose is relatively common for applications in LC/MS, using both packed beads (Martin et al., 2000; Young et al., 2007) and integrated porous monolith fashioned from polymer (Peterson et al., 2003) or silica (Luo et al., 2007). Nanoelectrospray emitters have also been used to produce integrated microreactors as well, such as the wall-functionalized tip of Zhao et al. (2006) or the online digestion/SPE/LC systems of Peterson et al. (2003) or Hedstrom et al. (2007).

The electrospray characteristics of functionalized or packed emitters are not substantially different from their unfunctionalized counterparts, and thus they will not receive much attention in this review. There are two main considerations, however. The first is the application of voltage which, as mentioned above, can be done either by coating the distal end of the integrated column/emitter with a conductive material or through the working solution *via* a precolumn electrode. The latter approach is somewhat complicated when the column length is very long since the solution resistance becomes important and when the solution composition (and thus its resistance) changes significantly during a run, which is common for many column techniques. The wire-in-tip method, on the other hand, becomes altogether inconvenient when solid material is present in the tip. The other main consideration is how the presence of solid material at the tip exit affects the spray, since it is now clear that tip geometry is of paramount importance in nanoelectrospray. Typically, unless the material is very near the exit aperture, there is little effect. However, if the material constricts flow from the opening the net effect is that the tip behaves more like one with a smaller i.d., being able to achieve lower flow rates (and hence smaller droplets) without the increased risk of clogging.

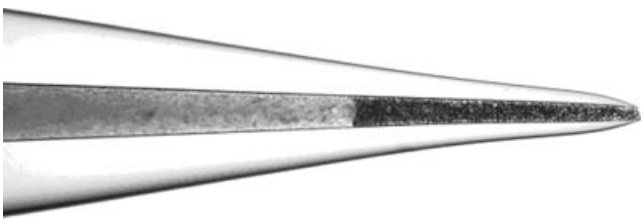

FIGURE 6. Optical image of a tapered emitter containing a silica frit that has been packed with chromatographic material to make an integrated column. Image courtesy of New Objective, Inc.

B. Multinozzle Emitters (Emitter Arrays)

1. Multiplexed Emitters for Sequential Measurement

Another consideration for the development of useful nanoelectrospray emitters is their integration into devices that include sample preparation and analysis steps for online analysis and MS detection. On a small scale these are called micro-total analysis systems (μ-TAS) or Lab-on-a-Chip devices, and research on such systems is still vibrant. The manufacture of these high-precision microfluidics devices has been facilitated by microfabrication techniques like photolithography, wet etching and laser ablation. The coupling of microfluidic devices to mass spectrometry has been a particularly active research area over the last two decades and various interfaces have been developed. Koster and Verpoorte (2007) have recently published a thorough review on the approaches used to couple these devices to MS and so we shall not dwell on this area. However, some devices have been developed that incorporate multiple nanoelectrospray emitters into a single device, which is a feature that we feel is a very important development in nanoelectrospray research. One reason for integrating multiple emitters is for improving the efficiency of the device by multiplexing analyses without having to change any components or to avoid cross-contamination of the emitter. For instance, Xue et al. (1997) developed a glass microchip with nine microfluidic channels (60 μm × 25 μm) by photolithography, wet etching and thermal bonding procedures, where multiple electrosprays were generated from the blunt outlet of the channel which had been chemically treated to attenuate wetting effects from spraying aqueous solutions. The chip (Fig. 7) was operated at a flow rate of 100–200 nL/min and used successfully in the detection of myoglobin down to low nanomolar concentrations.

Liu et al. (2000) further developed a prototype polymer-based multichannel device compatible with the standard microtitre well plate technology. The individual samples were driven to manually inserted capillary emitters (26 μm i.d.) *via* the 96 microchannels connecting each well by pneumatic assistance from nitrogen gas, and positioned relative to the MS orifice by a computer-controlled translation stage. This created a highly versatile system (Fig. 8) that alleviates sample contamination issues and was amenable to high-throughput analysis. In addition, the chip was made from an inexpensive plastic material and hence could be discarded after use, further diminishing sample carryover problems common with flow injection analysis. However, the developed multiemitter microchip consisted of the manual insertion of conventional electrospray tips into microfluidic channels which was not only cumbersome and prone

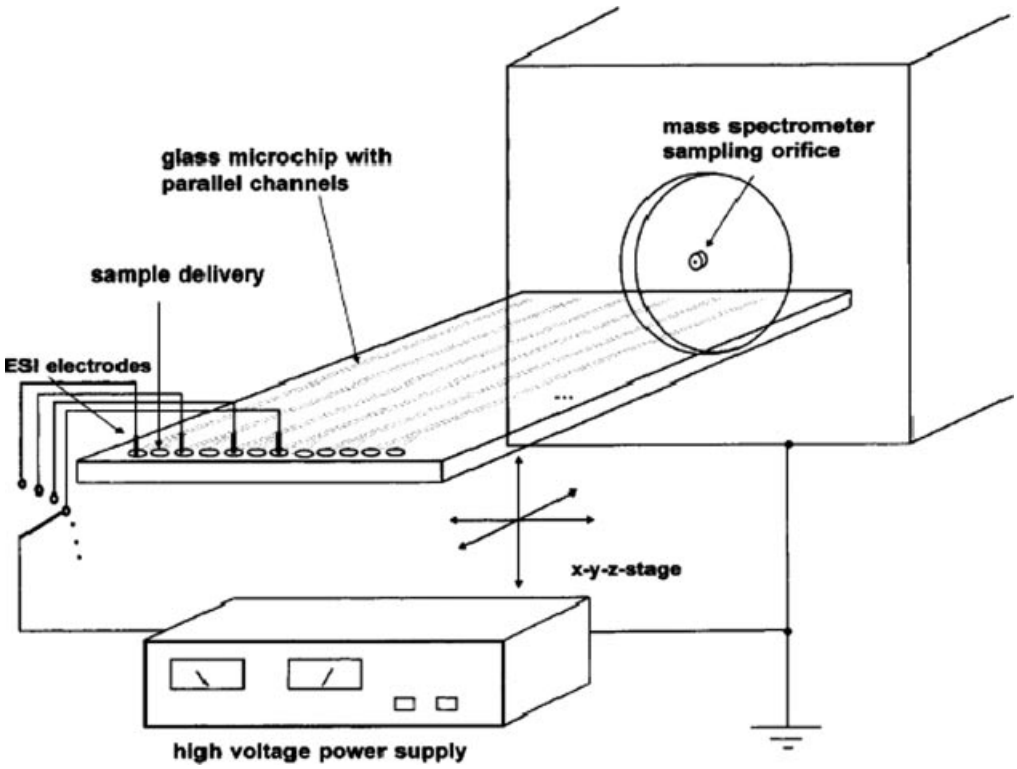

FIGURE 7. Schematic of a multichannel microchip spraying from the blunt end. Reproduced with permission from Xue et al. (1997). Copyright 1997 American Chemical Society.

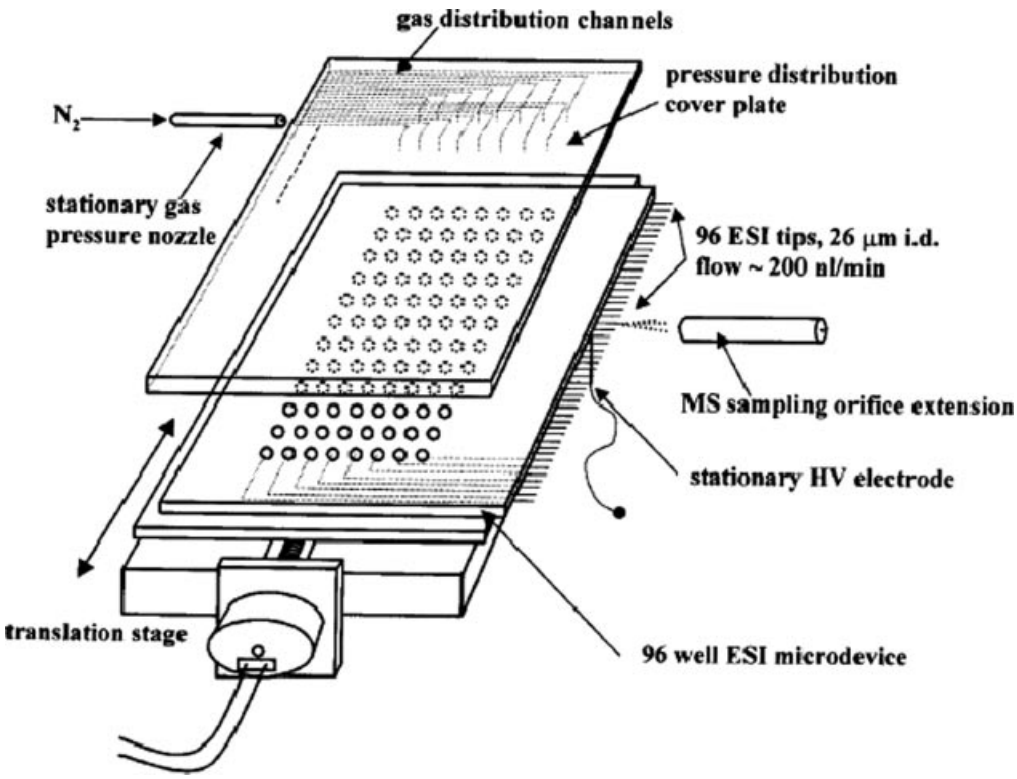

FIGURE 8. Schematic of a multichannel device with an array of electrospray emitters corresponding to all 96 wells of a microtitre plate for high-throughput MS. Reproduced with permission from Liu et al. (2000). Copyright 2000 American Chemical Society.

to clogging of both tips during operation and microchannels during the gluing step, but the approach did not lend itself to mass fabrication.

Other multiplexed electrospray platforms have been developed, including an array made of cyclic olefin copolymer chip-based porous polymer monolith (PPM) for solid phase extraction (SPE) and electrospray (Tan, Benetton, & Henion, 2003). The microchannel arrays were fabricated by capillary imprinting using a hot embosser. In this case, an array of capillaries (360 μm o.d., 50 μm i.d., 85 mm length) corresponding to the PPM columns was used for sequential electrospray.

Dayon et al. (2006) also reported a multiplex design which they called a multitrack electrospray chip (MTEC). They fabricated the chip by UV-photoablation using an excimer laser on a polyethylene terephthalate substrate. The MTEC consisted of up to six channels (length 1–1.5 cm, interchannel distance of 4–8 mm) with the distal end of the chip cut in a "V" shape to form a nozzle (100 μm × 40 μm) for electrospray (Fig. 9). The MTEC was placed at a fixed position of 1.5–2 cm relative to the MS orifice. Using this device up to six protein and peptide samples could be sequentially infused into a mass spectrometer, rendering the device highly suitable for fast screening applications in analytical chemistry and proteomics. As a bonus, the chips are reusable after a wash.

Kim and Knapp (2001) have also demonstrated a relatively straightforward microfabricated PDMS multiemitter for ESI-MS, which involved the formation of a PDMS microchip by photolithography followed by soft lithography. They produced microchips with one to four microchannels (100 μm × 30 μm), the exit apertures of which were shaped to a point at the edge of the chip. Wetting effects normally present at the blunt end of a chip were effectively alleviated due to the geometry and the hydrophobicity of the PDMS material. The chips for electrospray were tested by flowing angiotensin and bradykinin solutions at 3 μL/min (i.e., not nanoelectrospray) and the electrospray was found to be modestly stable but not as good as for tapered tips, with standard deviations ranging from 2.8% to 12.5%. Furthermore, the chips had an impressive lifespan of more than 30 hr. While their design was quite simplistic they demonstrated the possibility of interfacing multichannel-based microchips with simply designed integrated emitters. PDMS-based microchips, however, might be ineffective for biological assays due to the hydrophobic nature of the material which exacerbates non-specific adsorption of biomolecules, especially proteins (Sia & Whitesides, 2003). Also there is a limitation on the choice of solvents that are compatible with PDMS, since many organic solvents dissolve oligomers and additives that could give a large background in the resulting mass spectra. Other multiplexed electrospray systems have been reviewed in the article by Koster and Verpoorte (2007). It should be emphasized that in these cases the devices act as multiplexed electrospray sources, where individual emitters electrospray sequentially and not simultaneously and thus there is no specific sensitivity increase. However, the microfabrication techniques for these devices, and in some cases the devices themselves, are relevant to the development of simultaneous multi-ESI-MS.

FIGURE 9. Photograph of a multitrack electrospray chip with six channels. Reproduced with permission from Dayon et al. (2006). Copyright 2006 Wiley Interscience.

2. Multinanoelectrospray Emitters for Simultaneous Measurement

A key drive in ESI-MS research and development is the need for sensitivity, especially in the rapidly growing field of proteomics. Sensitivity enhancement has mainly been achieved by the increase in ionization efficiency, mostly by reducing the flow rate to the nano regime concomitant with reducing the size of the emitter aperture to create smaller charged droplets. However, a more recent approach has been to integrate multiple emitters generating multiple electrosprays, which leads to an increase in the spray current. Theoretically, spray current is increased by a factor of the square root of the number of electrosprays relative to a single emitter at the same flow rate (Tang et al., 2001) (Eq. 1). The concept of multiple electrospray has been used in other applications related to aerosol science (Salata, 2005). Building on the multiple electrospray concept to further increase sensitivity, Tang et al. (2001) microfabricated a polycarbonate chip using an excimer laser ablation technique. The nine-emitter array was arranged in a three-by-three configuration (Fig. 10) with an inter-emitter spacing of 1.1 mm and an orifice diameter of 30 μm. The incoming flow is split so that the flow rate to each individual emitter is reduced, favoring an increase in sensitivity. Using this emitter array similar electrospray signal stabilities were generated when compared to standard fused silica capillaries (100 μm i.d. and 200 μm o.d. tapered to 50 μm) at an infusion rate of 3 μL/min (i.e., 333 nL/min per emitter).

The ionization performance of this system was evaluated using two detection schemes. The first scheme employed a triple-quadrupole MS with a modified curtain gas skimmer and electrodynamic funnel to improve ion collection and transmission, and the second utilized an electrometer connected to a counter-electrode to monitor total spray current. The emitter configuration produced a two to three times gain in sensitivity at 1–4 μL/min flow rates, which was commensurate with theoretical predictions and suggests that each emitter sprayed independently (Fig. 10b). Interestingly, stable cone-jet mode electrosprays were only generated at abnormally high

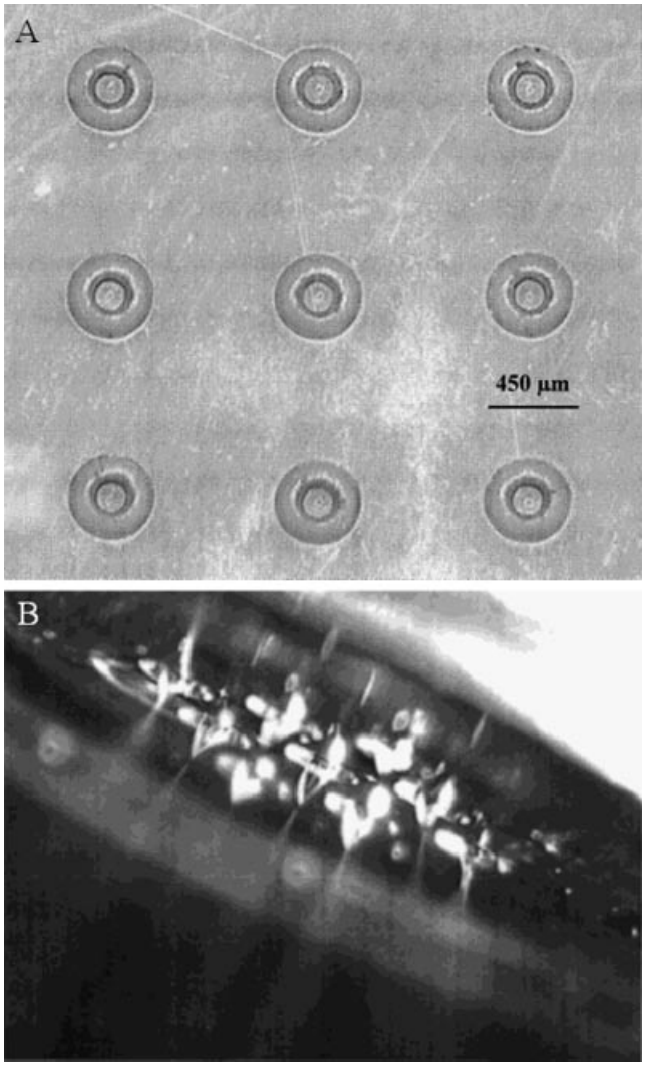

FIGURE 10. **A**: Three-by-three configuration of an emitter array made with polycarbonate using laser ablation; (**B**) Photomicrograph of nine stable electrosprays generated from the nine-emitter array. Reproduced with permission from Tang et al. (2001). Copyright 2001 American Chemical Society.

voltages near 7 kV. The configuration employed did not enable each individual emitter to be optimized for maximum ion current, however this could potentially offer a further increase in total ESI-MS signal and dynamic range. Furthermore, the larger emitting region requires the redesign of the MS ion guides and ion optics.

Schultz et al. (2000) used deep reactive ion etching (DRIE) to machine nozzles (approximately 15 μm deep) from the planar surface of a silicon wafer making a mass producible, low volume (<25 pL) and robust electrospray device (Fig. 11). The nozzles produced comparable electrospray stability (less than 4% RSD) to standard tapered emitters with good internozzle reproducibility (12% RSD for 10 nozzles). Schultz et al. (2000) demonstrated the merits of a micromachined nanospray nozzle and pointed to the application of micromachining for

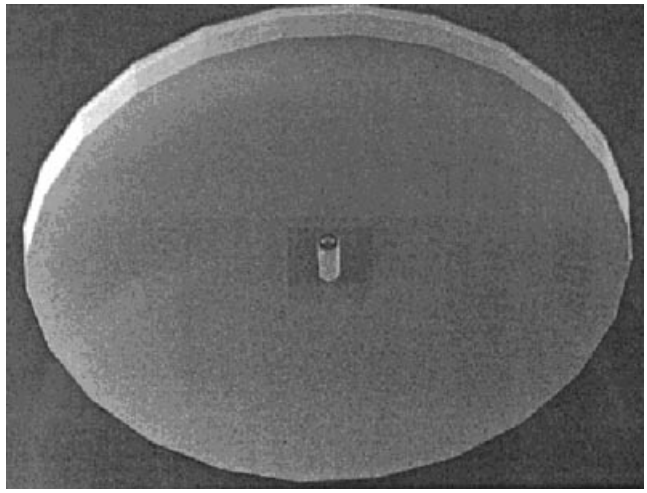

FIGURE 11. Electrospray nozzle microfabricated from silicon. Reproduced with permission from Schultz et al. (2000). Copyright 2000 American Chemical Society.

dense nozzle arrays. A similar micromachined nozzle array, Nanomate™ has been commercialized by Advion, Ithaca, NY, USA, Biosciences. Kim et al. (2007) employed MEMS fabrication methods for the fabrication of a "microfabricated monolithic multinozzle" (M^3) emitter (Fig. 12). Using this fabrication strategy they produced an array of 10 protruding nozzles (2 μm × 8 μm, width × depth, protruding 150–250 μm), with an internozzle distance of approximately 10 μm, in the cross sectional area of 100 μm × 8 μm of a main channel, producing a density of 100 nozzles/mm. Electrical contact was maintained using aluminum tape adhered to the conductive silicon.

A single nozzle M^3 emitter produced total-ion-count (TIC) traces with typical stabilities of RSD 4.5% while sensitivity and resolution were found to be comparable to standard pulled emitters when a standard peptide sample composed of Glu-fibrinopeptide B and bovine serum albumin was infused at 600 nL/min. As a result of their silicon composition, M^3 emitters required an elevated applied voltage (4.5–4.8 kV) to generate ESI compared to the commercial tapered tips (2.1–2.4 kV). Although this could be alleviated by metallizing the nozzles, the higher voltages are not unusual with multi-emitters. The requirement for higher applied potentials can be attributed to electric shielding, which becomes more severe with an increase in the number of emitters in the array (Tatemoto et al., 2007).

A slight increase in sensitivity for a five-nozzle tip was reported although sensitivity was not examined in any significant depth nor was the MS source/ion optics combination modified for the array. A larger array was not studied but clearly this could be accomplished in a reasonably facile fashion given current silicon micromachining capabilities. Deng et al. (2006) produced a 91-nozzle system using a similar fabrication strategy. Their study examined the relative effect of flow rate on droplet size, and showed that uniform droplets could be produced by parallelized electrosprays. Perhaps more importantly, each sprayer was operating in cone-jet mode.

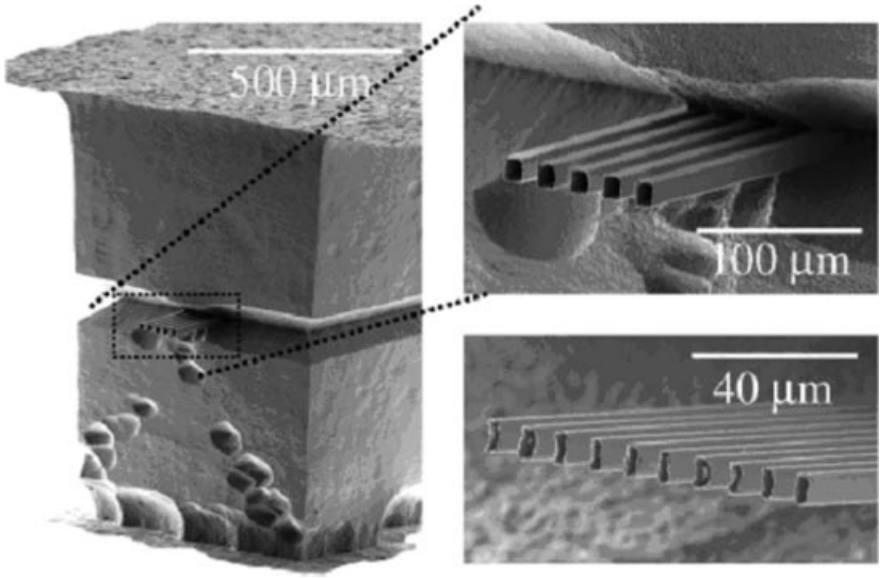

FIGURE 12. SEM image of the microfabricated monolithic multinozzle (M^3) emitter fashioned from silicon. Reproduced with permission from Kim et al. (2007). Copyright 2007 American Chemical Society.

An interesting alternative approach developed by Nissila et al. (2007) and Sainiemi et al. (2008) used a lidless micropillar array electrospray ionization chip (µPESI) fabricated from silicon using deep reactive ion etching (DRIE). The µPESI chip contained an array of micropillars with diameter, height, and distance between pillars in the range of 15–200 µm, 20–40 µm, and 2–80 µm, respectively, as shown in Figure 13a. This device provided a reliable and operationally simple open-channel filling based on capillary forces and a sharpened electrospray emitter tip for ionization. The microchip could be fabricated in a small footprint as shown in Figure 13b, where the µPESI is superimposed on a 10 euro cent coin. The microchip device produced a stable electrospray (<5% RSD) with continuous infusion. Additionally, the µPESI showed good quantitative linearity and high sensitivity, with a limit of detection of 60 attomoles for verapamil measured with tandem MS.

Microfabricated pillars have been postulated to be an ''ideal'' chromatography platform because of the ability to almost perfectly order the micropillars (stationary phase) through micromachining which minimizes the negative effects of eddy diffusion (Eijkel, 2007). The µPESI could potentially be useful for incorporating a chromatographic step and hence advance the potential of chip-based HPLC systems which are still in the early stages of development.

Another multi-emitter methodology that has been investigated involves the splitting of fluidic flows using a porous material placed at the exit aperture of the capillary or microfluidic chip. An embodiment of this method is a microchip hydrophobic membrane-assisted emitter, which features a polytetrafluoroethylene (PTFE) membrane with an average pore size of 0.22 µm thermally bonded to the exit channel aperture (100 µm width × 30 µm depth) of a polycarbonate microchip fabricated by hot embossing (Fig. 14) (Wang et al., 2004). The membrane-assisted platform was not only found to be quite effective in alleviating the liquid spreading at the exit of the microchannel because of its hydrophobic nature, but it also afforded stable electrospray at standard applied potentials (3.7 kV) and flow rates as low as 10 nL/min. The thermal bonding steps, however, tended to collapse some of the pores present in the membrane and clogging was also observed after a few hours of use. Using a similar approach, Su and Oleschuk (2008) showed a straightforward procedure to glue polysulfone and polycarbonate (PC) membranes with pore sizes in the range 0.2–5.0 µm to both a PC microchip and to 75 µm i.d., 360 o.d. fused silica capillary. While the approach is facile and was demonstrated to be effective in enabling nanoelectrospray and minimizing the spreading of droplets at the exit, robustness and batch fabrication would pose problems.

Another approach has been the *in situ* polymerization of a porous polymer at the exit aperture of the emitting capillary (Koerner et al., 2004; Lee et al., 2005). The fabrication strategy involved the photo-patterning of a porous polymer monolith (PPM) by the UV initiation of the pre-polymer mixture that consisted of an acrylate-based monomer and cross linker, porogenic solvent and the initiator. The polymer was formed in a UV-transparent capillary in a matter of minutes. The pores present in the PPM were found to be highly efficient for use as electrospray emitters (Fig. 15a). This class of emitters enabled stable electrospray over a wide range of flow rates (20–1,000 nL/min) and was found to resist clogging. This enhanced clogging resistance results from the multiple fluidic paths within the porous polymer.

At lower flow rates (<100 nL/min), rather than a single Taylor cone being observed a ''mist'' was generated and attributed to the formation of multiple Taylor cones resulting from the pore structure of the PPM (Fig. 15b). Additionally, the hydrophobic nature of the PPM limited the band broadening problems associated with droplet spreading at the exit of a typical silicate capillary due to silanophilic effects. Another key advantage of the PPM emitter platform is its ability to be

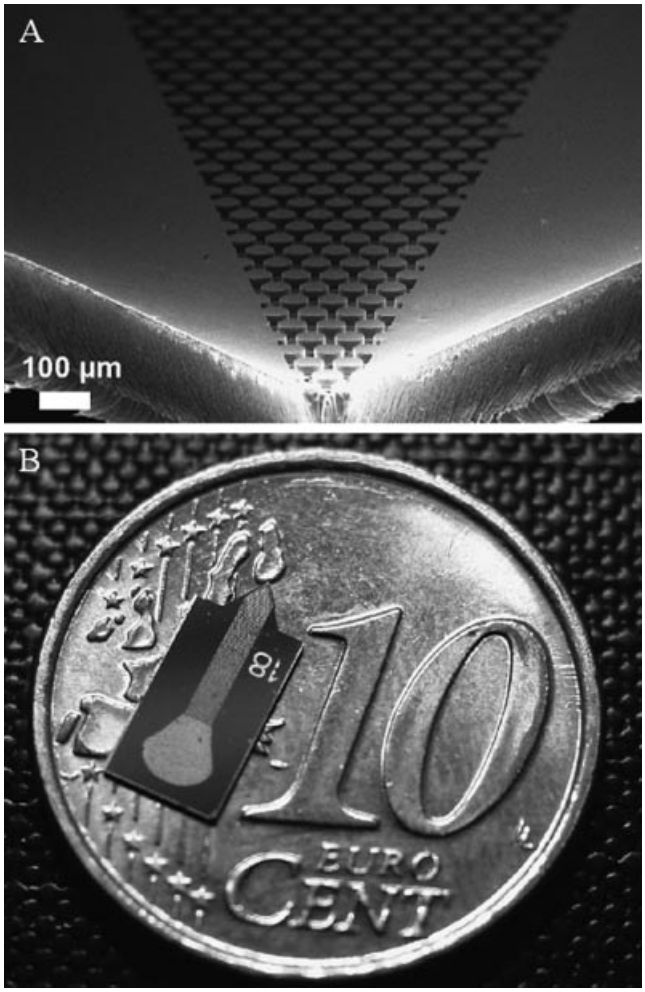

FIGURE 13. **A**: Emitter tip of the micropillar ESI chip (µPESI); (**B**) µPESI chip compared to a €0.10 coin. Reproduced with permission from Sainiemi et al. (2008). Copyright 2008 Elsevier.

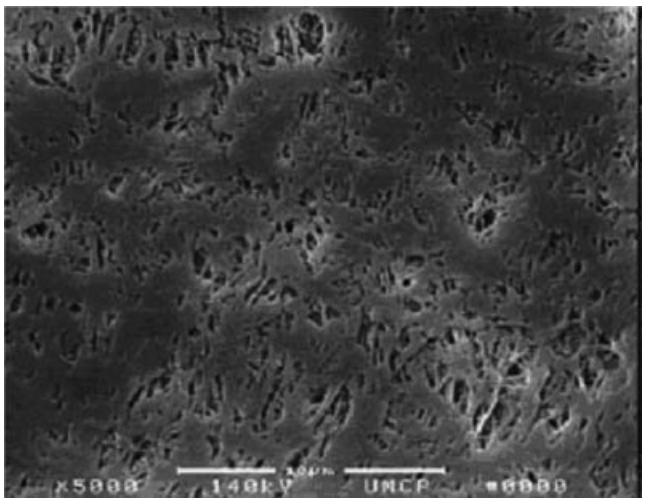

FIGURE 14. SEM image of a PTFE patch bonded to the exit surface of an electrospray chip. Reproduced with permission from Wang et al. (2004). Copyright 2004 Royal Society of Chemistry.

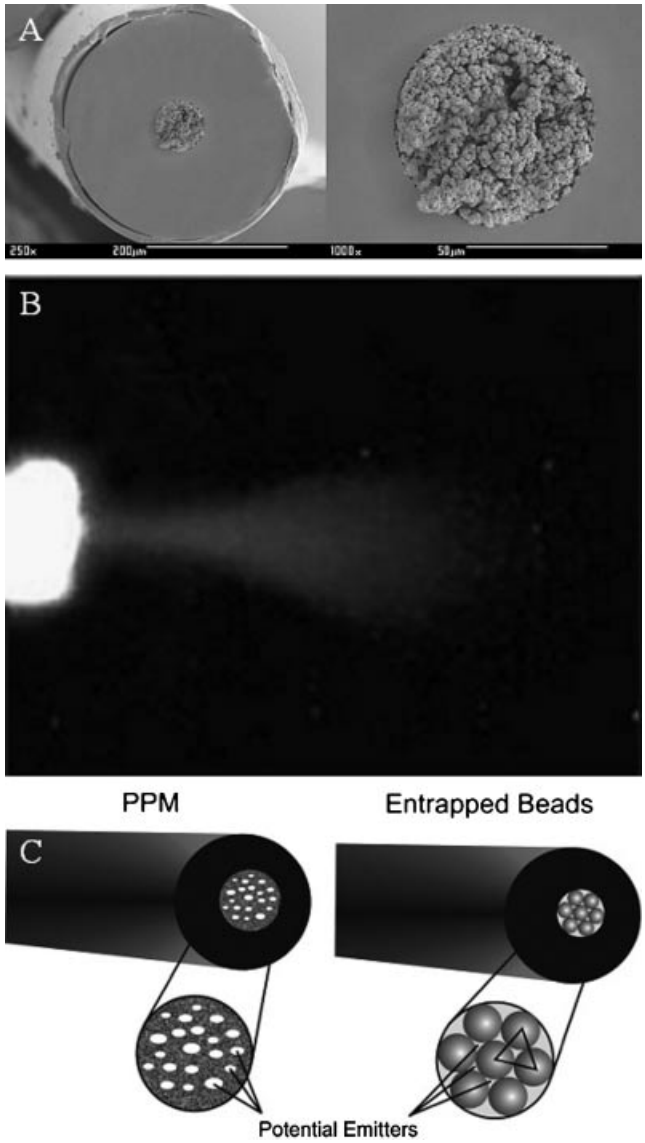

FIGURE 15. **A**: SEM image of a porous polymer monolith (PPM)-assisted ESI emitter; (**B**) Electrospray "mist" emanating from the PPM emitter at flow rates <100 nL/min. Reproduced with permission from Koerner et al. (2004). Copyright 2004 American Chemical Society; (**C**) Schematic comparing emitter exits from a PPM-assisted emitter and a polymer-entrapped microsphere emitter. Reproduced with permission from Koerner et al. (2007). Copyright 2007 American Chemical Society.

monolithically integrated with other sample preparative methodologies (e.g., capillary LC or SPE columns) on the same platform. Luo et al. (2007) fabricated a nanobore silica-based monolithic column that they used both as a separation column as well as an ESI emitter. The tapered PPM-filled tip was found to be similar in sensitivity and electrospray stability even at very low flow rates (10 nL/min) compared to the commercial tapered tip. Bedair et al. utilized a lectin-functionalized PPM to selectively preconcentrate and elute a glycosylated peptide prior to MS analysis. The PPM enables integrated sample preparation with no

column dead volume, high resistance to clogging and good run-to-run reproducibility. Because of the ease of PPM formation even in the confines of a microchip and its attractive use as a separation phase, PPM has been demonstrated to be an effective approach to interface microchip devices to mass spectrometry (Koerner & Oleschuk, 2005; Bedair & Oleschuk, 2006a,b). Another class of closely related emitters involves the entrapment of reversed phase microspheres with a butyl acrylate-based PPM matrix (Fig. 15c) (Koerner et al., 2007; Gibson et al., 2008). Instead of relying upon the chaotic pore structure developed during polymer synthesis, entrapped microspheres produce a material whose pores are the interstices between the uniformly-sized microspheres. As with the PPM based emitters, sample preparation could be integrated with a careful choice of microsphere and entrapping chemistry.

To further improve the multi-emitter platform, Kelly et al. (2007) developed a low-dead-volume (~200 nL) multi-emitter device specifically designed to be coupled with capillary LC. This device consisted of a linear array of 19 fused silica capillaries (19 μm i.d., 150 μm o.d.) arranged with a uniform interspatial distance of 500 μm center-to-center, aligned in space with the help of an aluminum holder (Fig. 16). The capillaries were bundled together and fed through a 1.5 cm-long PEEK tubing sleeve. The array of silica capillaries were etched together to provide externally tapered, evenly aligned emitters to ensure uniform performance (Kelly et al., 2006) The fact that the emitters are not tapered internally and that relatively large orifices can be used (typically 20 μm i.d.) alleviates clogging problems common with internally tapered emitters providing extended emitter lifetime.

The multi-emitter was found to increase the spray current commensurate with theoretical predictions (Eq. 1). With the increased spray current, however, using the standard mass spectrometer single inlet would limit the ion transfer efficiency. To ensure that the increased number of ions is efficiently transmitted to the mass spectrometer, the inlet was modified to consist of a linear array of 19 (400 μm i.d.) stainless steel capillaries corresponding to the multi-emitter array (Fig. 16). Using this configuration, the transfer efficiency was calculated to improve about fivefold compared to the single emitter/single inlet platform. To demonstrate that the increased transmitted ion current translates to enhanced sensitivity in the mass spectrometer, various concentrations of reserpine (100 nM to 10 μM) were analyzed by both the standard and multi-emitter/multi-capillary inlet configurations. The electrospray stability was indeed better (RSD 3.3%) for the multi-emitter compared to the standard single emitter (RSD 4.6%), which can be attributed to the statistical offset of the stability fluctuations of the individual emitters in the array resulting in improved stability of the measured signal. Ultimately, a sensitivity increase of approximately ninefold was achieved. Interestingly, the sensitivity gain was greater than the ion current gain, which can be attributed to improved desolvation resulting from the smaller charged droplets generated by the nanoelectrospray from the multi-emitter and hence with the efficient ion transmission platform most of the ions reach the detector. Additionally, the increased sensitivity with the multi-emitter operating in the nanoelectrospray regime could also be partially attributed to the placement of the emitter array tips very close (1.5 mm) to the modified MS inlet, further reducing ion losses. To further attest to the improved ESI-MS performance of the multi-emitter device, a complex peptide mixture of an albumin tryptic digest was tested and a significant sensitivity gain ranging from a factor of 2.4 to 12.3, depending on the peptide species, was observed. The wide range in sensitivity gain can be attributed to the innate differences in ionization efficiencies of the various analyte species. Furthermore, the significant increase in sensitivity even for complex analyte matrices shows minimized ion suppression due to the ability of the multi-emitter to operate within the nanoelectrospray regime. Higher emitter densities in a linear array configuration would feasibly increase the sensitivity, which was suggested would be possible by using capillaries having smaller outer diameters. The use of narrower bore capillaries should be useful in reducing the dead volume of the emitter arrays.

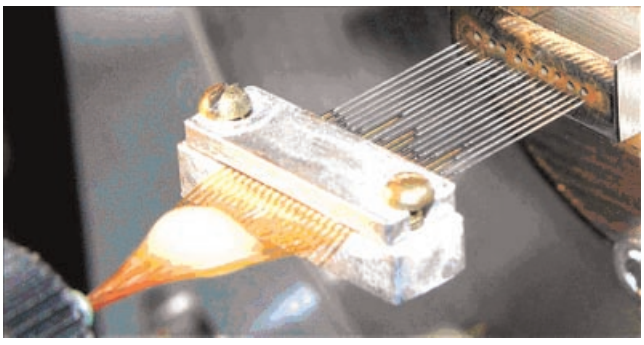

FIGURE 16. Linear array of 19 chemically etched capillary emitters with a corresponding MS inlet. Reproduced with permission from Kelly et al. (2008a). Copyright 2008 American Chemical Society. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The multicapillary emitter device was assessed for chromatographic coupling (Kelly et al., 2008a). In an attempt to enhance ion transmission, the MS inlet was modified to create larger multiple inlets, (to maximize ion collection by the MS), while decreasing the total length of the capillary inlets. The multi-emitter with modified inlets was compared to a single emitter/single inlet configuration by analyzing a tryptic digest of depleted human plasma separated on a reversed phase column at a flow rate of 2 μL/min. For both sources an applied ESI voltage of 2.0 kV with 1–1.5 mm spacing from emitter to inlet gave optimal measured ion currents. The intensities of individual peptides from spiked proteins were identified and compared using both platforms and, on average, a more than 11-fold increase was observed for the multi-emitter. The increased sensitivity compared to their previous report was attributed to the improved ion transmission efficiency as a result of the improved MS inlet architecture. While postcolumn band broadening is not severe with the multi-capillary emitter platform, it still inevitably degrades the ultimate fidelity of the chromatography, especially if lower flow rates are used and if any flow inhomogeneities exist between each of the emitter capillaries.

Another significant challenge with a planar capillary multi-emitter is the potential for electric field inhomogeneity among the emitters due to shielding effects, especially when they are closely

spaced. As such, the emitters at the periphery experience higher electric fields than the interior emitters for the same applied voltage and hence the emitters function in different regimes of the voltage versus electrospray profile. This problem may ultimately limit the ability to develop multi-emitters with a large number of emitters because the severity of the shielding effect is proportional to the number of emitters. These effects have been studied particularly in the application of multi-emitter platforms in other disciplines (Regele et al., 2002; Deng et al., 2006; Deng & Gomez, 2007), such as for microthrusters, thin film deposition and other aerosol-based applications. A possible solution to electric shielding as demonstrated by Deng et al. (2006) would be to contour the counter-electrode or the emitters in the array such that the interior emitters are closer to the counter-electrode, thus increasing the electric field at the center of the array and compensating for the shielding. Shielding can also be somewhat mitigated by minimizing the emitter-MS inlet spacing, although reducing this distance is deleterious to droplet desolvation especially at higher flow rates. To obviate the shielding problem, Kelly et al. (2008b) further modified the multi-emitter array so that the emitter capillaries were placed in a circular arrangement (Fig. 17) to enable all the emitters to operate in a similar electric field environment. While the circular capillary multi-emitter did not minimize shielding itself, the format was found to minimize the non-uniformity in electric fields experienced by the individual emitters in the array, enabling all the electrosprays to operate in the same regime. Correspondingly, the MS inlet was modified to match the circular emitter array format to ensure as high an ion transmission as possible. The signal enhancement for this format, however, was found to be modest and not as pronounced as the linear multi-emitter array.

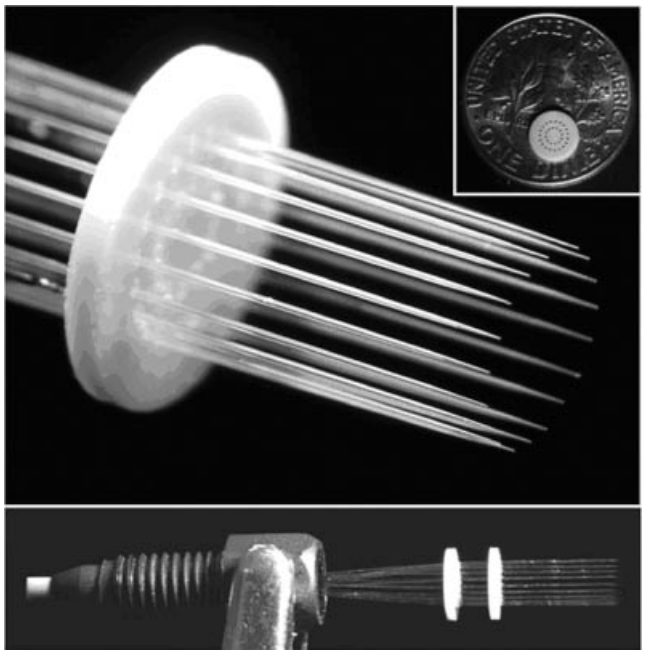

FIGURE 17. Circular array of chemically etched emitters to improve electric field homogeneity. Reproduced with permission from Kelly et al. (2008b). Copyright 2008 American Chemical Society.

This decreased ion transmission is thought to be due to an increase in space-charge effects at the greater ESI currents, leading to larger diffusional losses that compromise ion sampling by the capillary multi-inlet. A promising ion sampling strategy uses sub-ambient pressure ionization with nanoelectrospray source (SPIN) in which the electrospray is operated inside a 30 Torr MS inlet chamber that contains an electrodynamic funnel (Page et al., 2008). It is hypothesized that by electrospraying directly into the ion funnel, which captures and refocuses the entire ion plume, transmission losses would essentially be eliminated.

The density of emitters in this arrangement is limited due to the o.d. of the emitting capillaries. As an alternative, we have recently examined the use of microstructured fibers (MSF) as nanoelectrospray emitters. MSF's represent a relatively new class of silica optical fiber that confines light through the use of a microstructured cladding (Russell, 2003). The microstructured fibers are commercially produced by stacking silica capillaries to a desired preform, followed by heating and pulling (a technique commonly referred to as ''stack and draw''). The microstructured cladding is composed of a large array of 2–5 μm holes whose pattern can be precisely controlled during the manufacturing process. We have recently utilized a microstructured fiber with 30 independent fluidic channels (4–5 μm) for nanoelectrospray (manuscript in preparation), consistent with the typical aperture sizes of tapered emitters used for flow rates as low as a few 10's of nL/min (Fig. 18). The MSF emitter generated stable electrospray at flow rates from 10 to 500 nL/min with more significant sensitivity gains at low nano flow rates ($\leq$20 nL/min). For this 30-hole MSF, a 20 nL/min flow rate corresponded to a 66 pL/min flow rate per fluidic channel.

The silica-based MSF emitters can also be modified with typical silylation reagents to improve their nanoelectrospray characteristics. With simple chemical modification of the MSF emitter exit using chlorotrimethylsilane (CTMS), the emitters could effectively electrospray 99.9% aqueous samples with

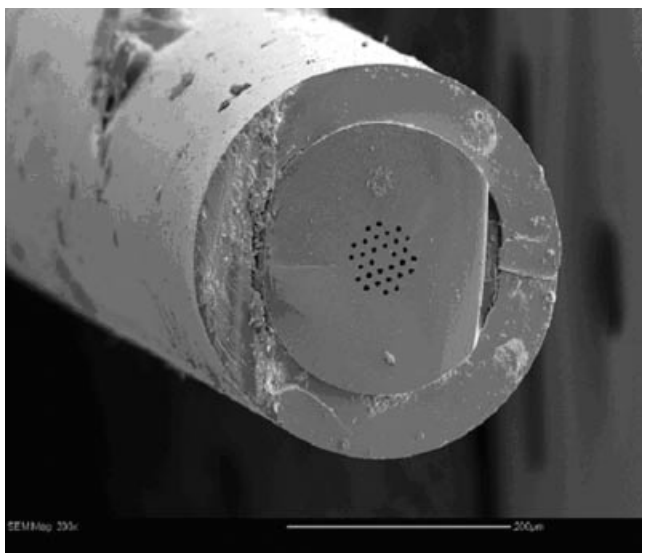

FIGURE 18. SEM image of a microstructured fiber (MSF) emitter having thirty 4–5 μm holes (magnification 200×, scale bar is 200 μm).

remarkable stability (RSD <6.0%) and sensitivity. The nanoelectrospray emitter density is significantly higher with this emitter type where 30 individual fluidic paths are confined to a 2,800 μm^2 cross-sectional area, which should enable a much larger number of Taylor cones and increased sensitivity. Furthermore, the dense emitter arrangement may produce emitter cross-talk and produce true multi-emitter behavior only with specific solvent compositions due to surface wetting effects while still being subject to shielding effects. Moreover, the larger ionization plume may affect the MS sampling efficiency resulting in lower sensitivity enhancements than expected. It is therefore envisioned that MSF emitters used in conjunction with an electrodynamic ion funnel would further increase sensitivity.

III. CONCLUSIONS AND PERSPECTIVE

Nanospray ESI-MS has not yet enjoyed the same success and robustness as its higher flow rate analogue, which in large part has stemmed from the emitter characteristics. The prevailing mood within much of the ESI-MS community is that only those who absolutely require the enhanced sensitivity associated with nano-ESI-MS use it. This has generally meant that most nanospray practitioners have been proteomic researchers whose samples are extremely limited in quantity and concentration. However, an additional driving force is appearing on the horizon that will seek to further reduce costs associated with analytical testing, as well as to develop ''greener'' analysis methods. Shifting to low-flow nano-LC (i.e., 500 nL/min) consumes a fraction of the solvent that is required for conventional milliliters per minute HPLC analysis, thus reducing solvent use and associated disposal costs. If one envisions the banks of HPLC units running constantly in pharmaceutical facilities alone, the saving becomes considerable. However, for the mainstream analytical community to adopt a low-flow separation paradigm, similar analytical performance, sensitivity and robustness must be demonstrated. Analytical performance will be less of an issue with the advent of smaller particle size columns and improved pumping systems capable of generating stable flow as low as a 10 nL/min. Sensitivity will continue to be a concern for proteomic practitioners, although those comfortable with current sensitivity at higher flow rates will certainly see improvements by operating in the nanoelectrospray regime. Single-aperture tapered emitters remain the most commonly used emitters for nanoelectrospray because of their ease of fabrication and commercial availability. Multi-emitters show promise for increased sensitivity, although some current designs are cumbersome and not amenable to mass production. An additional challenge in their utility is the possible requirement for a corresponding multi-MS inlet and newly designed ion optics, which could increase instrument costs considerably. Multi-emitters are currently in their infancy, but as the multinano-electrospray process is more fully understood, higher density arrays can be constructed. As a result, lower flow rates per channel can be achieved providing further increases in sensitivity while maintaining fluidic flow rate capacity. It is clear that nanoelectrospray emitters will continue to be an important component in the coupling of low-flow separation methods with mass spectrometry, and that efforts to improve cost and environmental friendliness, sensitivity and robustness will drive further improvements in emitter design.

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