Published in final edited form as:

J Am Soc Mass Spectrom. 2014 December ; 25(12): 2028–2037. doi:10.1007/s13361-014-0856-5.

Improving the Sensitivity of Mass Spectrometry by Using a New Sheath Flow Electrospray Emitter Array at Subambient Pressures

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Abstract

Arrays of chemically etched emitters with individualized sheath gas capillaries were developed to enhance electrospray ionization (ESI) efficiency at subambient pressures. By incorporating the new emitter array in a subambient pressure ionization with nanoelectrospray (SPIN) source, both ionization efficiency and ion transmission efficiency were significantly increased, providing enhanced sensitivity in mass spectrometric analyses. The SPIN source eliminates the major ion losses of conventional ESI-mass spectrometry (MS) interfaces by placing the emitter in the first reduced pressure region of the instrument. The new ESI emitter array design developed in this study allows individualized sheath gas around each emitter in the array making it possible to generate an array of uniform and stable electrosprays in the subambient pressure (10 to 30 Torr) environment for the first time. The utility of the new emitter arrays was demonstrated by coupling the emitter array/SPIN source with a time of flight (TOF) mass spectrometer. The instrument sensitivity was compared under different ESI source and interface configurations including a standard atmospheric pressure single ESI emitter/heated capillary, single emitter/SPIN and multi-emitter/SPIN configurations using an equimolar solution of 9 peptides. The highest instrument sensitivity was observed using the multi-emitter/SPIN configuration in which the sensitivity increased with the number of emitters in the array. Over an order of magnitude MS sensitivity improvement was achieved using multi-emitter/SPIN as compared to using the standard atmospheric pressure single ESI emitter/heated capillary interface.

INTRODUCTION

Although electrospray ionization (ESI) operating in atmospheric pressure is highly effective in generating multiply charged gas phase ions for analysis by mass spectrometry (MS), there is a significant ion transmission efficiency limitation at the MS inlet capillary/orifice interface [1, 2]. Analyte losses occur in large part because the ES plume covers a larger geometric area than the inlet capillary can effectively sample, such that only a fraction of the generated current is transmitted from atmospheric pressure to the first vacuum region of the mass spectrometer [3–5]. Previous attempts to increase ion transmission efficiency at the ESI-MS interface include using a multi-capillary inlet [6, 7] or less effectively by increasing

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the size of the inlet aperture [8]. However, substantial losses still occur [2], particularly for higher flow rate electrosprays that must be displaced at a greater distance from the inlet. Additional attempts to improve ion transmission from ambient pressure into the first vacuum stage of the mass spectrometer also include inlet ionization techniques where ionization occurs in the inlet capillary itself rather than at an emitter tip thus removing losses to the front of the inlet capillary[9–11].

An approach under extensive investigation in our lab involves removing the inlet interface conductance constraint completely and incorporating the ESI source directly inside the first lower pressure chamber of the mass spectrometer [12–14]. Coined subambient pressure ionization with nanoelectrospray (SPIN), this approach places the ESI emitter adjacent to a low capacitance ion funnel in a subambient pressure environment. Under this configuration, the entirety of the spray plume can be sampled into the ion funnel and losses associated with ion transfer from ambient pressure into the first vacuum region are essentially eliminated. A SPIN/dual ion funnel interface was developed recently to effectively transmit the analyte ions from ESI source to MS detector [15].

The SPIN source is conceptually similar to a previously developed electrohydrodynamic ionization technique which operates at much lower pressures [16]. This method was shown effective with nonvolatile liquids including glycerol [16], liquid metals [17, 18], and ionic liquids [19] at low flow rates. However, studies conducted with caffeine[20, 21] at low pressures (<1 Torr) suffered from poor performance due to liquid boiling, droplet freezing, and inefficient solvent evaporation. The SPIN source overcomes these issues by operating at significantly higher pressures (e. g. 10–30 Torr) and incorporating a heated CO₂ desolvation gas and a high speed sheath gas to increase charged droplet desolvation and electrospray stability [14]. At low liquid flow rates (e.g. 50 nL/min) as much as 50% of ion utilization efficiency was demonstrated by the single emitter/SPIN source which essentially implies that one in every two analyte molecules initially in the sample solution is effectively converted to a gas phase ion and transmitted through the interface into the high vacuum region of the mass spectrometer [22]. The ion utilization efficiency increases as the flow rate decreases suggesting that higher desolvation and ionization efficiency can be achieved for the smaller charged droplets at SPIN source operating pressures [23, 24].

However, the capability of operating electrospray in the nanoliter per minute flow rate range for optimum ionization efficiency is often limited by the need to online couple ESI-MS with liquid chromatography (LC) separations which operate at much higher liquid flow rates in general. An effective solution to solve this flow rate mismatching problem is the employment of an emitter array in the ESI source [25–27]. The use of ESI emitter array effectively splits the incoming large liquid flow, as required by the LC separation, into an array of nano flow rate electrosprays for high ESI efficiency. In addition, the total ESI current generated at a given flow rate was shown to be proportional to the square root of the number of emitters allowing “brighter” ion sources [28]. The combined smaller charged droplets and high electric current has made the emitter array a promising ESI source for high sensitivity MS [25–34] and a variety of other applications such as nanoparticle synthesis [35], space propulsion [36], and microcombustion [37]. In the case of utilizing emitter array for MS analysis it is possible to tune the fabrication to further increase emitter density, but it

is all of marginal benefit if the additional current generated cannot be efficiently delivered into the low pressure region of the mass spectrometer. To this end, combining the benefits of an emitter array with the SPIN source is potentially of great interest.

Here we report the development of a new emitter array/SPIN source for high sensitivity MS. The new emitter array design uses an equal number of sheath gas capillaries concentric to each emitter in the array to allow the generation of stable multi-electrosprays at subambient pressures for the first time. The motivation to include individual sheath gas capillaries for the emitters originated from our early SPIN source performance evaluation that showed a significantly improved electrospray stability and droplet desolvation efficiency by incorporating a sheath CO₂ flow around the ESI emitter [14]. The spatial current profile of the ES plumes in an emitter array was measured in great detail to demonstrate the stability and uniformity of the electrosprays generated by the emitter array. MS analyses using a mixture of 9 peptides were performed to compare sensitivity under different SPIN source configurations involving the use of different numbers of emitters in the arrays. The overall sensitivity of the emitter array/SPIN-MS was also evaluated against the sensitivity of the standard atmospheric pressure single emitter/ single heated capillary inlet-MS. Additionally the size of the conductance limiting orifice of the high pressure ion funnel was increased to accommodate the larger currents generated by emitter arrays and further improve ion transmission efficiency. The physical mechanism behind the observed sensitivity improvement by using emitter arrays in the SPIN source was further probed by measuring the fraction of gas phase ion current in the total spray current generated by the arrays of different emitter numbers at the exit of the SPIN source.

EXPERIMENTAL SECTION

Sample Preparation

The ESI solvent consisted of 0.1% formic acid (FA, Sigma-Aldrich, St. Louis, MO) in 10% acetonitrile (ACN, Fisher Scientific, Pittsburgh, PA) and deionized water (Barnstead Nanopure Infinity System, Dubuque, IA). Stock solutions of 9 peptides (human angiotensin I, human angiotensin II, bradykinin, fibrinopeptide, kemptide, melittin, neurotensin, porcine angiotensinogen, and substance P, all purchased from Sigma-Aldrich) were prepared in the ESI solvent. Aliquots from the stock solutions were mixed and diluted to a final concentration of 1 μ M for each peptide.

Fabrication of Emitter Arrays with Individualized Sheath Gas Capillaries

The new emitter array mainly consisted of two types of fused silica capillaries, smaller one (150 μ m o.d., 10 μ m i.d., Polymicro Technologies, Phoenix, AZ) for the ESI emitters and the concentric larger one (360 μ m o.d., 200 μ m i.d., Polymicro Technologies) for sheath gas around the emitter. The completed emitter assembly and a step by step schematic of the emitter fabrication are shown in figure 1a. In the first step a sheath gas capillary preform was constructed by inserting the larger fused silica capillaries of roughly 10 cm length through a PEEK sleeve (0.055 in. i.d., 1/16 in. o.d., Upchurch Scientific, Oak Harbor, WA). To arrange the capillaries into a desired circular array of 4, 6, or 10 emitters, their distal ends were inserted into a 0.5 cm-diameter PEEK disk spacer with 400 μ m diameter holes

arranged in two concentric circles of 3.50 and 5.08 mm in diameter respectively. For example, to achieve a 6 emitter array with uniform spacing between the emitters the capillary tubes were inserted into every other hole in the inner circle of the spacer.

In the second step the capillaries were fixed in place by epoxy (HP 250, ITW Devcon, Danvers, MA) at the interior end and at the distal end behind the spacer. After the epoxy was cured the interior end is cut with a rotary tubing cutter. In the third step the preform was inserted into a T-junction and fixed into place with a ferrule nut. An additional piece of PEEK tubing (0.030 in. i.d., 1/16 in. o.d., Upchurch Scientific) was inserted into the opposite end of the T junction and held into place with an additional ferrule nut. The emitter capillaries were threaded through the preform and the rest of the assembly to protrude roughly 1–2 cm from the second PEEK sleeve. The emitters were sealed into position with epoxy at the second seal and the residual ends of the capillaries were cut off with a rotary tubing cutter after the epoxy had cured restricting the liquid flow to the emitter capillaries only. In the final step the polyimide coating was removed in a solution of NanoStrip 2X (Cyantek, Fremont, CA) at 100° C for 25 minutes and the emitters were chemically etched[38] in a solution of 49% HF (Fisher Scientific, Fair Lawn, NJ) to form externally tapered emitters of uniform length. Etching of the emitter inner wall was avoided by pumping water through the emitter array at a flow rate of 100 nL/min per emitter during the etching process. A photograph and photomicrograph of the completed emitter assembly is found in the supplemental material.

Electrospray Current Measurement and Profiling

The electrospray current was measured by positioning the emitter array with a three axis translational stage (Newport, Irvine, CA) perpendicular to a counter electrode [28]. The solution was infused through a transfer capillary by a syringe pump (model no. 22, Harvard Apparatus, Holliston, MA) from a 100 μ L syringe (Hamilton, Las Vegas, NV). The ESI voltage was applied to the stainless steel union connecting the transfer capillary to the emitter array by using a high voltage dc power supply (model no. PS350, Stanford Research Systems, Sunnyvale, CA). The current was measured using a picoammeter (model no. 6485, Keithley, Cleveland, OH) in line with the counter electrode. Current values were obtained from 100 consecutive measurements which were averaged using the built in data acquisition procedure of the picoammeter. The current transmitted through the high pressure ion funnel was measured by using the low pressure ion funnel as a charge collector with the DC voltage lines connected to the picoammeter.

A specially designed linear electrode array was used to spatially profile the electric current distribution of the entire plume generated by the ESI emitter arrays. This electrode array consisted of 23 electrically isolated Kapton-coated copper wires (340 μ m o.d.) in a line with equal spacing and incorporated into a conventional MS inlet capillary assembly as described in detail previously [2]. The front of the inlet was machined flat and polished with 2000 grit sandpaper to make the ends of the wires flush with the metal surface. The other ends of the wires were connected to an electrical breadboard with one connection to the common ground and the other to the picoammeter. The ES current was profiled in the horizontal dimension by sequentially measuring the current from each wire electrode in the array using

the picoammeter. To obtain the profile in the vertical dimension the multi-emitter arrays were raised incrementally by the translational stage at a fixed distance between the emitter array and the linear electrode assembly.

Mass Spectrometry

The performance of the emitter arrays was evaluated using an orthogonal TOF MS instrument (model no. G1969A, Agilent technologies, Santa Clara, CA) equipped with a dual ion funnel interface [7]. The conventional atmospheric pressure capillary inlet was replaced by the SPIN source which has been described in detail previously [12, 22]. In this configuration the ESI emitter array is placed inside the first vacuum chamber containing a low capacitance ion funnel. A diagram of the instrumental set up is shown in Figure 1b. Mass spectra for the ESI and SPIN configurations were acquired in the 200–1000 m/z range in positive ESI mode with 1 s TOF acquisition time. In our standard funnel interface design, a DC-only electrode with a 2.5 mm conductance limiting orifice diameter was used between the high pressure and low pressure ion funnels. This standard orifice diameter was used in all the experiments unless specified otherwise when the effect of the conductance limit orifice diameter on the ion transmission efficiency through the high pressure ion funnel was studied (Figure 5).

RESULTS AND DISCUSSION

We first sought to characterize the electrospray generated from the emitter arrays at ambient pressure. Figure 2a shows the two-dimensional ion current profile obtained from an array of six emitters. The distance between the emitter and the counter electrode was 2 mm. A solution of 0.1% FA in 10% ACN and deionized water was directly infused into the emitter array at a total flow rate of 100 nL/min. The ion current profile shown in Figure 2a demonstrates two distinctive features of the multi-electrosprays including: 1) the electrospray plumes generated from six emitters are independent from each other (no overlap of adjacent plumes); and 2) the size of the six plumes is very similar to each other.

One of the difficulties in the fabrication and operation of emitter arrays is the non-uniform electrical shielding effect that arises from interference from adjacent emitters and inhomogeneous electric fields. By arranging the emitters in a circular geometry the electric field experienced at each individual emitter will be uniform and independent of the emitter position in the array as long as the distance between emitters is constant [31]. The similar electrospray plume structure for all six electrosprays shown in Figure 2a confirms that the non-uniform electric shielding effects for our circular emitter array are minimal and that the electrospray generated from each emitter is in a similar ESI regime at a given electrospray voltage. This is indicated by the similar plume size and the ion current value measured between roughly 3–4 nA in the core of the each individual plume. From the 2-dimensional profile it is evident that the plume area from each emitter in the array is similar and independent. A radial ion current profile across two electrospray plumes, as shown by the inset in Figure 2a, further confirms the similarity of the electrosprays both of which demonstrate good axisymmetric current profiles with a similar width of 2.5 mm at an emitter distance of 2 mm from the counter electrode.

The ion current profile shown in Figure 2a also implies that a special MS inlet will need to be developed in order to effectively sample the large ion current produced by the ESI emitter array into the mass spectrometer if the emitter array is operated at atmospheric pressure. The optimum inlet design would be a complimentary multi-capillary inlet with its geometric configuration matching that of the emitter array so that each electrospray in the array aligns with an inlet capillary in the multi-capillary inlet. This becomes impractical as the number of ESI emitter increases, and finite pumping limitations will constrain the overall ion transmission efficiency [2, 30]. These challenges can be effectively resolved by coupling emitter arrays with the SPIN source to completely remove the capillary inlet. In this manner the ES plume is directly sampled in the SPIN source and can be effectively focused and transmitted by the ion funnel into MS analyzer with high efficiency [22].

Figure 2b shows the total ion current measurements at different electrospray voltages for different emitter arrays. In each case the total flow rate was 100 nL/min. The spray current obtained from a 4, 6, and 10 emitter array were compared to the measured current from a single emitter at the same total ESI flow and voltage. At any given voltage the total ESI current increases as the number of emitters increases. The ion currents measured with the 4, 6, and 10 emitter arrays were on average 2.1 ± 0.1 , 2.3 ± 0.1 and 2.8 ± 0.2 times larger than the corresponding current generated with a single emitter. This is consistent with existing theory that the ion current increases proportionately to the square root of the number of electrosprays relative to a single emitter at the same total flow rate [28]. From these results we demonstrate that a larger current can be generated from an emitter array at a given voltage compared to a single emitter.

Our early attempts to utilize emitter arrays in the SPIN source proved unfruitful due to wetting of the outer surface of the emitters in the array, leading to an unstable and non-uniform array of electrosprays. By incorporating individualized sheath gas capillaries for each emitter in the new emitter array design, we were able to effectively solve the emitter wetting problem and establish stable array of electrosprays at subambient pressures. To demonstrate the ESI stability at subambient pressures a solution containing equimolar concentration of 9 peptides was infused continuously at a total flow rate of 200 nL/min over a period of 5 hours with no change in the instrumental set up. Figure 3a shows the extracted ion currents (EIC) from the neurotensin +3 charge state, angiotensin I +3 charge state and angiotensinogen +3 charge state from a 5 minute data acquisition obtained from a 6 emitter array. Figure 3b shows the average magnitude of the same peptide EICs taken from individual 5 minute traces acquired over the five hour duration. The trace shown in Figure 3a was taken from the initial 5 minute acquisition. The signal for the 6 emitter array displayed little variability over time with roughly 1.4% relative standard deviation (RSD) which was similar to the 2.2% RSD observed with a single emitter (data not shown). Over the 5 hour duration (Figure 3b) the overall ion intensity varies 8%, 11% and 12% for neurotensin, angiotensin I and angiotensinogen indicating excellent spray stability. The peak intensity ratio for the two most abundant peaks, angiotensin I +3 and neurotensin +3, was 0.60 ± 0.02 during the initial 5 minute run and remained unchanged throughout the entire 5 hour duration. Similar ESI stability was observed also for the 4 and 10 emitter arrays used for this study.

The benefit of the higher ESI current generated by the ESI emitter array is further demonstrated by the representative mass spectra collected from the 9 peptide mixture for various emitter arrays in Figure 4. A constant sample infusion rate of 200 nL/min was used for all emitter array configurations. The mass spectra were obtained from a 1 s acquisition time averaged over 1 minute. The instrument parameter settings including all ion funnel rf and dc voltages were kept constant as the conditions in the low capacitance ion funnel were previously characterized and optimized for interface with the SPIN source [12, 22]. The instrumental parameter that seemed to exhibit the greatest effect on MS signal was the SPIN source operating pressure. The spectra in figure 4 were obtained at the corresponding optimum operating pressures for different emitter arrays and applied voltage was adjusted for each emitter array to obtain the optimal signal. Specifically, the optimum pressure for a single emitter was roughly at 16 Torr whereas the optimum pressure decreased to ~14 Torr for a 4 emitter array and ~11 Torr for both the 6 and 10 emitter arrays. The lower optimal pressures observed for larger number emitter arrays may arise from the increased solvent vapor pressure due to the higher droplet evaporation rate by the smaller droplets generated by the larger emitter array that requires larger pumping speed (e.g. lower chamber pressure) to maintain efficient droplet desolvation. The light blue curve shows the spectra obtained using a conventional atmospheric pressure ESI source with a single emitter, a heated capillary inlet, and a dual ion funnel interface. Figure 4 clearly demonstrates that the SPIN source significantly improves analyte signal intensity as compared to the conventional ESI source and the signal intensity also increases as the number of ESI emitters in the SPIN source increases. The zoomed in spectra for the neurotensin and angiotensinogen +3 ions in the insets of Figure 4 quantitatively show that the neurotensin and angiotensinogen peak intensity increases by a factor of 13.5 and 4.2 respectively when a single emitter in the SPIN source is compared to the conventional single emitter ESI source. Additionally, by comparing a single emitter in the SPIN source to an emitter array under the same conditions the neurotensin peak increases by factors 1.7, 2.0 and 2.7 and the angiotensinogen peak increases by 2.0, 2.7, and 4.0 for a 4, 6, and 10 emitter array, respectively. This correlates to observed enhancement factors of 23.1, 26.3 and 35.7 for the neurotensin peak and 8.7, 11.5 and 17.1 for the angiotensinogen peak with 4, 6, and 10 emitter arrays relative to a single emitter conventional ESI interface.

A summary of the enhancement factors observed for the most abundant charge state from each peptide in the 9 peptide mixture is shown in Table 1. The average enhancement factor obtained from a single emitter and 4, 6, and 10 emitter arrays in the SPIN source relative to a conventional single emitter ESI source were 6.6, 11.2, 13.3, and 15.1 respectively. The data in Table 1 also indicate that the signal enhancement for higher charge state peptides is more pronounced than the enhancement for peptides in lower charge states using the emitter array. For example, signal enhancement for the melittin +4, angiotensinogen +3 and neurotensin +3 ions is significantly larger than that for substance P +2, angiotensin II +2, and bradykinin +2 ions. Even for these latter peptides, a higher enhancement factor was observed in their higher charge states. The greater signal enhancement for higher charge state peptides using an emitter array is most likely due to the greater ion currents generated by the emitter arrays supplying more abundant excess charges for producing higher charge state peptide ions.

Additional gains in sensitivity can also be achieved by increasing the conductance limiting orifice of the high pressure ion funnel. Figure 5 shows mass spectra acquired from the 9 peptide mixture using a 10 emitter array in the SPIN source at conductance limit orifice diameters of 2.5 mm (black trace), 3.0 mm (blue trace) and 4.0 mm (green trace), respectively. The inset of figure 5 further shows the ion current transmitted through the first ion funnel at each corresponding conductance limit orifice diameter. The mass spectra shown in figure 5 illustrate that the average peptide ion abundance increased by a factor of ~2.6 by increasing the conductance limit orifice diameter from 2.5 mm to 4 mm. This enhancement factor is in addition to the aforementioned gains associated with using an emitter array in SPIN source relative to single emitter ESI demonstrating further gains in MS sensitivity. Enhancement factors for the predominant peptide peak from the 9 peptide mixture at the 4.0 mm diameter conductance limit are shown in table 1. For every peptide in the mixture, except fibrinopeptide A and bradykinin, the signal intensity increased at least an order of magnitude relative to single emitter ESI with enhancement factors as high as 48.0 and 52.7 for melittin and neurotensin respectively. The inset in figure 5 also shows clearly that higher ion current can be transmitted through the high pressure ion funnel using a larger conductance limit orifice diameter at each electrospray voltage. This is especially relevant in the present case because in order to fully realize the potential gains associated with “brighter” ion sources such as an emitter array, it is necessary to increase the transmission characteristics of the ion funnel interface. Additional experiments are currently underway in our lab to explore the possibility of further increasing the conductance limit orifice to allow for even greater ion transmission efficiency through the high pressure funnel.

To further probe the ionization efficiency and the ion transmission characteristics in the SPIN source, ion current transmitted through the (first) high pressure ion funnel was measured under rf on and off conditions for different ESI emitter arrays. In this set of experiments, the (second) low pressure ion funnel was used as a charge collector. The DC voltage lines of the low pressure ion funnel were connected to a picoammeter for the transmitted current measurement. Assuming that the ion current measured under the rf off condition consists mostly of large charged clusters which are beyond the mass to charge (m/z) range detectable by the TOF MS and could not be effectively confined by the rf field applied to the ion funnel, the characteristic ionization efficiency in the SPIN source can then be defined as $Q_{\text{eff}} = (I_{\text{on}} - I_{\text{off}})/I_{\text{on}}$, where I_{on} is the current measured under rf on condition and I_{off} is the current measured under rf off condition. The difference in current, ($I_{\text{on}} - I_{\text{off}}$), represents largely the gas phase ions generated by the ESI process that can be both confined by the ion funnel rf field and transmitted through the SPIN source with high efficiency.

Figure 6 shows the current measurements at different ESI voltages under both high pressure ion funnel rf on and off conditions for a single emitter and a 10 emitter array in the SPIN source using the 9 peptide mixture. At an ESI voltage of 2.5 kV only 0.75 nA of current was transmitted through the funnel with the rf off for single emitter, whereas 1.62 nA of current was observed with rf on. This represents about 54% ionization efficiency according to the definition above (e.g. gas phase ions transmitted through the funnel contribute about 0.87 nA current). In contrast, 3.53 nA and 0.81 nA of current were measured using the 10 emitter array at the same ESI voltage with the rf on and off, respectively. The ionization efficiency

is increased to 77% at the same ESI flow rate implying a significant improvement of desolvation due to the smaller droplet sizes generated by the 10 emitter array. Over the entire range of tested electrospray voltages, the average ratio of gas phase ion current generated by using a 10 emitters array to that by using a single emitter is 3.00 ± 0.16 nA. This gain in gas phase ion current is also consistent with the MS ion intensity measurements shown in Table 1, which a factor of 2.3 increase in ion intensity using a 10 emitter array relative to the single emitter in the SPIN source for the same 9 peptide mixture sample.

The experimental data firmly indicate that even more signal enhancement can be obtained by further increasing the number of emitters. We are currently exploring alternative ways to fabricate high density ESI emitter arrays with individual sheath flow around each emitter. Although only a signal enhancement factor of 2–4 was observed using a maximum 10 emitter array in this study relative to a single emitter under the SPIN source operating pressures, the gain in MS sensitivity becomes much more pronounced when the emitter array in SPIN source is compared to a single emitter in the conventional ESI source. For example, the 10 emitter array in SPIN source exhibited an average enhancement factor of 15.1 and as high as a 30 fold increase was observed specifically for neurotensin and melittin ions. In addition, the average enhancement factor rose to 21.4 and as high as ~50 for melittin and neurotensin over conventional ESI by further increasing the conductance limit orifice diameter from 2.5 mm to 4.0 mm. The utility of this new emitter array/SPIN source for high sensitivity MS measurements are currently being further explored in the lab.

CONCLUSIONS

We presented a method to fabricate ESI emitter array with individual sheath gas flow around each emitter in the array. These emitter arrays exhibited excellent electrospray stability and uniformity. The spatial profile of the ESI plumes in two dimensions demonstrates that each emitter behaves independently from each other and the current measurements from the emitter array follow consistently with the well-established ESI theory. These emitters were incorporated in the SPIN source and their MS performance was tested systematically using a 9 peptide mixture. A signal enhancement correlative to the number of emitters in array was observed and a higher enhancement was observed for peptide of higher charge states. Of the current generated from the source a higher fraction of useful analyte ions was generated and transmitted through the ion funnel by the larger number emitter array demonstrating increased ionization efficiency. The combination of electrospray emitter arrays and the SPIN source constitutes a powerful tool for maximizing both ionization and ion transmission efficiency for ultrasensitive ESI-MS analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Portions of this research were supported by the National Center for Research Resources (RR18522), National Cancer Institute (1R33CA155252), National Institute of General Medical Sciences (8 P41 GM103493-10), the Laboratory Directed Research and Development Program at Pacific Northwest National Laboratory (PNNL), and by the Department of Energy Office of Biological and Environmental Research Genome Sciences Program under

the Panomics project. All the experiments were performed in the Environmental Molecular Sciences Laboratory, a U.S. Department of Energy (DOE) national scientific user facility located at PNNL in Richland, Washington. PNNL is a multiprogramming national laboratory operated by Battelle for the DOE under contract DE-AC05-76RLO01830.

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Figure 1. Fabrication of emitter arrays with individual sheath gas capillaries (a). See text for additional information. MS instrumentation configuration of the SPIN source interface coupled with an ES array positioned at the entrance of the low capacitance ion funnel (b).

Figure 2.

Two-dimensional ESI spray profile generated from a 6 emitter array infusing 0.1% formic acid in 10% acetonitrile at a flow rate of 100 nL/min. The distance between the emitter and the counter electrode was fixed at 2 mm (a). The lower right inset shows the one-dimensional current profile taken along the dotted line. Current vs. voltage curves for various emitter arrays under the same experimental conditions (b).

Figure 3. ESI-MS stability experiments. Extracted ion currents from selected analyte peaks obtained from a 6 emitter array during a 5 minute data acquisition (a) and incrementally over a 5 hour duration (b). The neurotensin +3 charge state is shown in black, angiotensin +3 charge state in red, and angiotensinogen +3 charge state in green.

Figure 4.

Mass spectra from the analysis of the 9 peptide mixture with various emitter arrays and single emitter configurations operated in optimized conditions. The total flow rate was 200 nL/min in each case and the concentration of each peptide in the mixture was 1 μ M. The insets show zoomed images of the mass spectra of the neurotensin +3 charge state (558.3 m/z) and angiotensinogen +3 charge state (587.0 m/z).

Figure 5.

Mass spectra from the analysis of 1 μ M 9 peptide mixture using a 10 emitter array in the SPIN source and conductance limiting orifice diameters of 2.5 mm (black trace), 3.0 mm (cyan trace), and 4.0 mm (green trace). The inset shows the current transmitted through the high pressure ion funnel measured at different electrospray voltages for the different conductance limit diameters.

Figure 6. ESI current transmitted through the low pressure ion funnel obtained from direct infusion of the 9 peptide mixture at a single emitter and 10 emitter array at a flow rate of 200 nL/min.

Table 1

Intensity ratios obtained from comparing the peak intensity of the most abundant charge state from the MS analysis of the 9 peptide mixture at a single emitter ESI interface with a heated capillary inlet to emitter arrays operated in the SPIN source*.

<i>m/z</i>	Charge State	Peptide	Single Emitter Spin	4 Emitter Array	6 Emitter array	10 Emitter Array	10 Emitter Array (CL 4.0)
768.9	2+	Fibrinopeptide A	1.7	2.8	3.1	4.2	6.5
712.2	4+	Melittin	12.9	25.5	30.9	34.1	48.0
674.4	2+	Substance P	8.1	13.0	14.0	9.5	12.9
587.0	3+	Angiotensinogen	4.2	8.7	11.5	17.1	22.4
558.3	3+	Neurotensin	13.5	23.1	26.3	35.7	52.7
530.8	2+	Bradykinin	3.9	5.3	6.7	6.2	9.4
523.8	2+	Angiotensin II	4.9	7.4	7.4	7.4	11.1
432.9	3+	Angiotensin I	5.9	9.3	12.6	14.1	19.4
386.7	2+	Kemptide	4.3	5.5	7.3	7.3	10.2
Average Enhancement Factor			6.6	11.2	13.3	15.1	21.4

* Except where noted all data was collected with a conductance limit in the high pressure ion funnel of 2.5 mm in diameter.