Published in final edited form as:

J Am Soc Mass Spectrom. 2015 January ; 26(1): 55–62. doi:10.1007/s13361-014-0998-5.

On the Ionization and Ion Transmission Efficiencies of Different ESI-MS Interfaces

Jonathan T. Cox, Ioan Marginean, Richard D. Smith, and Keqi Tang*

Biological Sciences Division, Pacific Northwest National Laboratory, P.O. Box 999, Richland, Washington, 99352

Abstract

The achievable sensitivity of electrospray ionization mass spectrometry (ESI-MS) is largely determined by the ionization efficiency in the ESI source and ion transmission efficiency through the ESI-MS interface. These performance characteristics are difficult to evaluate and compare across multiple platforms as it is difficult to correlate electrical current measurements to actual analyte ions reaching the detector of a mass spectrometer. We present an effective method to evaluate the overall ion utilization efficiency of an ESI-MS interface by measuring the total gas phase ion current transmitted through the interface and correlating it to the observed ion abundance measured in the corresponding mass spectrum. Using this method we systematically studied the ion transmission and ionization efficiencies of different ESI-MS interface configurations, including a single emitter/single inlet capillary, single emitter/multi-inlet capillary, and a subambient pressure ionization with nanoelectrospray (SPIN) MS interface with a single emitter and an emitter array, respectively. Our experimental results indicate that the overall ion utilization efficiency of SPIN-MS interface configurations exceeds that of the inlet capillary-based ESI-MS interface configurations.

INTRODUCTION

Electrospray ionization (ESI) has become the dominant ion source for broad areas of mass spectrometry (MS) applications due to its ability to generate multiply charged gas phase analyte ions and ease of coupling with online separation techniques such as liquid chromatography (LC) [1–4]. Operating electrospray in the nL/min flow rate range, referred to as “nanoESI”, improves the overall ionization efficiency compared to the higher flow rates more generally used [5–7]. In a conventional nanoESI source the emitter is typically positioned very close to (~2–3 mm) the sampling inlet of the MS, often a flow restricting heated capillary. Analyte ions generated from the highly charged droplets in the electrospray before or after entering the MS inlet are transmitted to the mass spectrometer for mass to charge (m/z) ratio measurements, where the achievable MS sensitivity is significantly determined by the transmission through the inlet capillary. A significant fraction of possible analyte ions are lost either due to the limited flow through the inlet, or to surfaces during their transit through the interface capillary or other apertures and conductance limits associated with the overall design [8, 9].

*Corresponding author: keqi.tang@pnnl.gov.

To improve ESI-MS interface designs, extensive studies have been performed on various processes occurring in the ESI-MS interface such as the mechanism of charged droplet formation in the electrospray [10–13] and the effects of liquid flow rate on droplet size and observed signal intensity [5, 7, 14–16]. Parallel to these studies, another method to increase the magnitude of current transmission is to use brighter ion sources such as an ESI emitter array which improves MS sensitivity [17–23]. However gains associated with improvements in ion sources are minimal if the increased current cannot be effectively transmitted through the ESI-MS interface. Due to this limitation, additional experimentation has focused on optimizing source geometries and interface conditions to improve ion transmission [8, 9, 24–29]. In an attempt to resolve the major ion transmission efficiency problem at the ESI-MS interface, a subambient pressure ionization with nanoelectrospray (SPIN)-MS interface was developed to remove the constraint of a sampling inlet capillary/orifice by placing the ESI emitter in the first MS vacuum chamber adjacent to the entrance of an electrodynamic ion funnel [30, 31]. Other approaches have demonstrated the utility of a hydrodynamic funnel shaped inlet capillary to increase the interface ion transmission efficiency [32]. Although this method does demonstrate improvements in transmitted ion currents from atmospheric pressure into vacuum, the contribution to the current from actual gas phase analyte ions cannot be segregated from residual solvent/cluster ions. Therefore measuring current alone may not completely reflect the efficiency of an ESI-MS interface.

The focus of this work is to evaluate the current transmission characteristics of an ESI-MS interface and provide a method to determine the ion utilization efficiency of various ion source and interface configurations. The ion utilization efficiency is defined in general as the proportion of analyte molecules in solution that are converted to gas phase ions and transmitted through the interface [31] and is determined by measuring the transmitted gas phase ion current through the interface and correlating it to the observed analyte ion intensity in the mass spectrum. Specifically, we systematically compared the transmitted gas phase ion currents through a heated inlet capillary ESI-MS interface and the SPIN-MS interface. Of all the interface configurations under investigation in this study, the highest transmitted ion current was measured by using the SPIN/ESI emitter array combination. We demonstrate that the SPIN-MS interface exhibits greater ion utilization efficiency than a conventional ESI-MS interface and furthermore that the ion utilization efficiency can be used as an effective metric to evaluate the overall performance of any ESI-MS interface design.

EXPERIMENTAL SECTION

Reagents

1 mg/mL stock solutions of each peptide (human angiotensin I, human angiotensin II, bradykinin, fibrinopeptide A, kemptide, neurotensin, porcine angiotensinogen, and substance P, all purchased from Sigma-Aldrich) were prepared in 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). A peptide mixture containing 10 μ M of each peptide was prepared from the stock solution in the same

solvent mixture. Prior to MS analysis the peptide mixture solutions with final concentrations of 1 μ M and 100 nM for each peptide were prepared by further dilution.

Mass Spectrometry

Mass spectra were acquired using an orthogonal TOF MS instrument (model G1969A, Agilent Technologies, Santa Clara, CA) with its standard interface replaced by a tandem ion funnel interface. Spectra were summed over 1 minute with a 1 s TOF acquisition time over a 200–1000 m/z range with a 0.1 m/z step size in positive ion mode. Unless otherwise noted, the high pressure ion funnel was operated with an variable RF peak to peak voltages of 300 V at frequency of 2.55 MHz and a DC gradient of 19 V/cm. The low pressure funnel was operated with an RF peak to peak voltage of 100 V at a frequency of 730 kHz and a DC gradient of 19 V/cm. A gate valve between the last high pressure funnel electrode and the DC-only conductance limit electrode was used to facilitate the change of ion source (i.e. from ESI to SPIN) without venting the instrument.

ESI-MS interfaces

Three different interfaces were used in this study including a) a single capillary inlet ESI-MS interface; b) a multi-capillary inlet ESI-MS interface and c) a SPIN-MS interface. A schematic of the capillary inlet ESI-MS and SPIN-MS interfaces is shown in Figure 1. The single inlet ESI-MS interface utilized a stainless steel capillary (7.6 cm long, 490 μ m i.d.) heated to 120° C and terminated roughly flush with the first high pressure ion funnel electrode. The multi-capillary ESI-MS interface consisted of seven 7.6 cm long and 490 μ m i.d. inlet capillaries arranged in a hexagonal pattern with one in the center. The multi-capillary inlet was also heated to 120° C with its exit flush with the first high pressure ion funnel electrode. In all the experiments using both the single capillary and the multi-capillary ESI-MS interfaces, the ESI emitter was mounted on a three axis translation stage and positioned roughly 2 mm in front of the capillary inlet. The SPIN-MS interface has been described in detail previously [30, 31, 33]. Briefly, the ESI emitter was placed inside the first vacuum region of the MS instrument via a vacuum feed through with the pressure adjusted in a range of 19–22 Torr. The emitter protrudes roughly 2 mm from a cylindrical outlet (5 mm in diameter) and is positioned on the axis of the high pressure ion funnel roughly 1 mm from its first electrode. The cylindrical outlet functions as the electrospray counter electrode and was biased 50 V higher than the front plate of the high pressure ion funnel. Sufficient droplet desolvation is accomplished by using a heated CO₂ gas (~ 160 °C) with its flow rate controlled by an Agilent G1995A flow meter. An additional CO₂ sheath gas was provided around the ESI emitter via a fused silica capillary (O.D. 360 μ m, I.D. 200 μ m) to ensure electrospray stability and prevent electrical breakdown.

All electrospray emitters were prepared by chemically etching fused silica capillaries (O.D. 150 μ m, I.D. 10 μ m, Polymicro Technologies, Phoenix, AZ) according to a previously described method [34]. The emitter arrays were prepared according to the procedures described in detail previously to include individual coaxial sheath gas capillary for each emitter in the array [33]. The emitter and emitter arrays were connected to a transfer capillary and a 50 or 10 μ L syringe (Hamilton, Las Vegas, NV) via two stainless steel unions. Solutions were infused with a syringe pump (model no. 22, Harvard Apparatus,

Holliston, MA) and ESI voltages were applied to the stainless steel union with a high voltage dc power supply (model HV-RACK-4-250-00229, Ultravolt, Ronkonkoma, NY).

Current Measurements

The gas phase ions that were transmitted through the high pressure ion funnel, were measured using the low pressure ion funnel as a charge collector by connecting the funnel DC voltage lines to a Keithley picoammeter (Model 6485, Cleveland, OH). Each current value reported was an average current from 100 consecutive measurements using the built in data acquisition function of the picoammeter. For clarity these measurements will be referred to as the electric current whereas the ion count measured by the mass spectrometer will be referred to as the total ion current (TIC) or extracted ion current (EIC) for a specific analyte, respectively.

RESULTS AND DISCUSSION

Characterization of ionization and ion transmission under different ESI-MS interface configurations

To probe both the ionization and ion transmission efficiencies under different ESI-MS interface configurations, the nature of the ion cloud in the high pressure ion funnel was systematically characterized. This was accomplished by measuring both the total transmitted electric current through the high pressure ion funnel, and the total ion current measured at MS detector for various interface configurations at different ion funnel RF peak to peak voltages, as shown in Figure 2. In general, the charged particles in the high pressure ion funnel contain both fully desolvated gas phase ions and the residue charged analyte/solvent clusters/particles. The portion of fully desolvated gas phase ions determines the final intensity of the ion current detected by MS, and the best sensitivity is achieved when this is maximized for any ESI-MS interface configuration. This portion of the ion cloud is also capable of being focused and transmitted with high efficiency by the high pressure ion funnel at sufficiently high RF voltage. In general, the fraction of the fully desolvated gas phase ions in the total transmitted electric current through the high pressure ion funnel would be determined by both the ionization/desolvation efficiency and the ion transmission efficiency in the ESI source and ESI-MS interface which also determines the overall MS sensitivity. Figure 2a shows the total electric current transmitted through the high pressure ion funnel at different RF voltages and under four different interface configurations, including a single emitter/single inlet capillary interface (black trace), a single emitter/seven inlet capillary interface (red trace), a single emitter/SPIN-MS interface (blue trace), and a 10 emitter array/SPIN-MS interface (green trace). The peptide mixture containing 100 nM of each peptide, as described in the experimental section, and a constant ESI infusion rate of 200 nL/min were used in all the experiments. Other funnel operating parameters (e.g. RF frequency, DC gradient, and chamber pressure) were held constant. The total transmitted electric current shown in Figure 2a increases monotonically with the increase of the high pressure ion funnel RF voltage. At a RF voltage of 300 V, the maximum RF voltage allowed before electric breakdown occurred, the maximum transmitted currents were measured at 0.54 nA and 0.66 nA for the single emitter/single inlet capillary interface and the single emitter/seven inlet capillary interface, respectively. In contrast, a much larger transmitted

current of 1.22 nA and 3.20 nA was observed for the single emitter/SPIN-MS interface and 10 emitter array/SPIN-MS interface, respectively. The significant increase in transmitted electric current through the high pressure funnel for both SPIN-MS interface configurations implies an improvement in the charge capture efficiency of the electrospray plume compared to the two heated capillary interfaces.

For comparison purposes and to correlate the transmitted electric current to MS signal, Figure 2b shows the extracted ion current (EIC) for the representative 3+ neurotensin ions ($m/z = 558.3$), the most intense peak in the mass spectrum, at different RF voltages for the same four interface configurations as Figure 2a. The other analyte peaks all exhibited similar sigmoidal trends with varying RF voltages. The measurements shown in Figures 2a and 2b indicate consistently that larger transmitted electric currents through the high pressure funnel would in general result in higher MS peak intensities under any interface configurations; with the 10 emitter array/SPIN-MS interface showing both the largest transmitted current and the highest MS peak intensity at any RF voltages among the four tested interface configurations in this study.

A noticeable difference between Figure 2a and Figure 2b is the different dependences of the transmitted electric current and MS peak intensity on the high pressure ion funnel RF amplitude (voltage peak-to-peak). At zero RF voltage, a baseline transmitted electric current was always measured while close to zero peak intensity was measured in the MS spectra for all the analyte ions, as represented by the intensity measurement of 3+ neurotensin ions in Figure 2b, in all four interface configurations. This is a strong implication that this baseline transmitted electric current dominantly consisted of large residue charged analyte/solvent clusters/particles undetectable by MS. As RF voltage increases, both the transmitted electric current and the analyte peak intensity increases initially. The increase of the analyte peak intensity is mainly due to the increase in the fraction of fully desolvated gas phase ions in the total transmitted electric current due to the increasing RF confinement provided by the ion funnel. Beyond 225 V RF voltage, the analyte peak intensity measurements in Figure 2b become maximized and essentially independent of RF voltage in the tested range of 225 V to 300 V voltages while the transmitted electric currents continue to increase. The fact that the measured analyte peak intensity plateaus is a strong indication that the RF confinement in the high pressure ion funnel is sufficient for all the gas phase analyte ions in the ion cloud beyond 225 V RF voltage. The continued increase of the transmitted electric current beyond 225 V RF voltage is likely due to the contribution of higher m/z small charged analyte/solvent clusters transmitted through the high pressure ion funnel under the increasing strong RF ion confinement, which do not contribute to detected ions.

From the transmitted electric current measurements in Figure 2a, the maximum gas phase ion current transmitted through the high pressure ion funnel can be further obtained by the difference of transmitted electric currents between $RF = 225 \text{ V}_{p-p}$ and $RF = 0 \text{ V}_{p-p}$ amplitudes based on the above discussion. The fraction of the gas phase ion current in the total transmitted electric current in any RF voltage can thus be well approximated by the current difference between RF on and off up to $RF = 225 \text{ V}_{p-p}$,

$$\Delta i = i_{RF,on} - i_{RF,off} \quad (1)$$

Using the above definition and from the experimental data in Figure 2a, the i between RF = 225 V_{p-p} and RF = 0 V_{p-p} was measured at 0.15 nA with the single emitter/single inlet capillary configuration. The i increased slightly to 0.20 nA with the single emitter/seven inlet capillary configuration. Significantly higher i measured at 0.44 nA and 1.32 nA were obtained for the single emitter/SPIN and the multi-emitter/SPIN configurations, respectively. The experimental data in Figure 2b also indicate a direct correlation between i and the analyte intensity in the mass spectrum up to RF = 225 V_{p-p} when all the gas phase ions in the high pressure ion funnel are effectively confined and transmitted. In the RF voltage range of 0 to 225 V_{p-p}, the significantly higher i shown in Figure 2a for both SPIN configurations as compared to both inlet capillary configurations directly translates into the higher ion intensity in Figure 2b. The magnitude of i at a given RF voltage thus offers a good measure for the efficiency of a given interface configuration which is determined by both the ionization efficiency and the ion transmission efficiency in the ion source and MS interface.

To further validate above claim that the measured i up to 225 V_{p-p} is a reasonable measurement of the total gas phase ion current produced in the ion source and transmitted through the high pressure ion funnel, the total ion current (TIC) at MS detector was systematically measured by using a direct infusion of the same peptide mixture as used in the Figure 2 experiment and the single emitter/SPIN configuration as the RF voltage was incrementally stepped down from 300 V_{p-p} to 0 V_{p-p}. As shown in Figure 3a, the TIC almost remains constant initially as the RF voltage steps down from 300 V_{p-p} to 225 V_{p-p} as a minor 15% reduction in TIC was measured. The majority of the decrease in TIC occurred when the RF voltage was further stepped down from 225 V_{p-p} to 0 V_{p-p} which is consistent with the intensity measurement of 3+ neurotensin ions in Figure 2b. To further clarify this, Figure 3b overlays the two mass spectra acquired at RF voltages of 225 V_{p-p} (black trace), and 0 V_{p-p} (red trace), respectively. While the peak intensities for all the analytes remain essentially constant between RF voltages of 300 V_{p-p} and 225 V_{p-p}, they drop by more than an order of magnitude from 225 V_{p-p} to 0 V_{p-p}. For example, the intensity of the neurotensin 3+ peak decreased only by 3% when the RF is decreased from 300 V_{p-p} to 225 V_{p-p}. The results in Figure 3 thus confirm the correlation between i and the total gas phase ion current transmitted through the high pressure ion funnel although the defined upper limit of RF 225 V_{p-p} is somewhat arbitrary. It is also expected that with sufficiently high RF voltage (>225 V_{p-p}) applied to the high pressure ion funnel the magnitude of the i would be largely determined by the ionization efficiency.

With the ability to correlate the measured i to total gas phase ion current in the high pressure ion funnel and MS TIC, additional experimental measurements were conducted to systematically compare the ionization efficiency under different interface configurations by measuring the i and corresponding analyte MS peak intensity at a constant high pressure ion funnel RF voltage of 225 V_{p-p} and different ESI liquid flow rates. The selection of the upper limit RF voltage in this set of experiments ensured that all the gas phase ions generated in the ion source were all transmitted through the high pressure ion funnel with

~100% ion transmission efficiency and the contribution from the small charged analyte/solvent clusters to i at an even higher RF voltage was minimized based on the experimental measurements shown in Figures 2 and 3. Figure 4 shows the i and the EICs for angiotensin II 2+ ($m/z = 523.8$), bradykinin 2+ ($m/z = 530.8$), and fibrinopeptide A 2+ ($m/z = 768.9$) at different ESI flow rates for a single emitter/single inlet capillary ESI-MS interface (a) and a single emitter/SPIN-MS interface (b), respectively. The results in Figure 4 show that i follows well with the analyte MS peak intensity in the entire ESI flow rate range from 20 nL/min to 1 μ L/min. Consistent with the typical ESI-MS characteristics [16], both the i and the analyte peak intensity initially increase as the ESI flow rate decreases as the ESI efficiency in both interface configurations is largely determined by the ionization and charged droplet desolvation efficiency. Further reducing the ESI flow rate from ~100 nL/min, the measured i and analyte peak intensity for both interface configurations decrease as the ESI flow rate decreases, consistent with ion current being limited by the availability at the given flow rate. The ESI flow rate effect on the MS sensitivity has been well documented and confirmed by several early studies [5, 7, 14–16, 35,36]. The results in Figure 4 further confirm that i can be used to both qualitatively and quantitatively correlate the analyte MS peak intensity. A factor of 3 to 4 increase in i for the single emitter/SPIN-MS interface (Figure 4b) as compared to the measured i for single emitter/single heated capillary ESI-MS interface (Figure 4a) corresponds to a similar analyte peak intensity improvement.

Determination of Ion Utilization Efficiency

Base on the well confirmed correlation between i and TIC, as discussed above, the ion utilization efficiency under the different ion source configurations can be evaluated by calculating the theoretical maximum analyte ion current that would be generated if all the analyte ions in solution were completely converted to the gas phase ions via the electrospray process and relating it to the experimentally measured i and TIC. For the theoretical maximum analyte ion current calculation, we begin with an individual analyte and assume that each compound can be generated into multiply charge states via electrospray. The theoretical maximum analyte ion current, I_J , if all molecules of a specific analyte J in the solution were converted to gas phase ions is given by:

$$I_J = QF \sum_{z=1}^{i(J)} z \delta_{z,J} C_J, \quad (2)$$

where Q is the liquid flow rate, F the Faraday constant, $\delta_{z,J}$ the fraction of compound J that carries z charges, C_J the molar concentration, and $i(J)$ the maximum number of charge carried by compound J [37]. Both $\delta_{z,J}$, and $i(J)$ can be determined experimentally based on the compound charged species (e.g. including different adduct ions and charge states) observed in the mass spectrum. The total maximum analyte ion current, I_A , for a solution containing N number of compounds if all the analyte molecules in solution are completely ionized is given by:

$$I_A = QF \sum_{J=1}^N \left(\sum_{z=1}^{i(J)} z \delta_{z,J} C_J \right). \quad (3)$$

By measuring the ion flux at the mass detector and the gas phase ion current transmitted through the interface, the ion utilization efficiency for a given MS interface configuration [6] can be evaluated. For an individual analyte the ion utilization efficiency, ϵ_J , for all four different interface configurations used in this study is given by:

$$\epsilon_J = \frac{\sum_{z=1}^{i(J)} \Delta i X_{z,J,TIC}}{I_J}, \quad (4)$$

where i is defined by eq. (1) and $X_{z,J,TIC}$ is the ratio of the extracted ion current for the z charge state of compound J to the total ion current observed from the mass spectrum. The overall ion utilization efficiency, ϵ_A of a given interface configuration for the analyte mixture can then be calculated by:

$$\epsilon_A = \frac{\Delta i X_A}{I_A} \quad (5)$$

where X_A is the ratio of total analyte current measured at the detector (by summing the EICs for all observable analyte charge states) to the TIC. For example, from the mass spectrum acquired from the infusion of 1 μ M peptide mix at 100 nL/min for the single emitter/SPIN configuration the charge state distribution, $\delta_{z,J}$, for angiotensin I is 0.08, 0.72 and 0.20 for the +4, +3 and +2 charge states respectively. The calculated theoretical maximum ion current, I_J , if all the angiotensin I molecules in solution when completely converted to gas phase ions is 0.46 nA from eq. (2). The ratio of the extracted ion current to the TIC, $X_{z,J,TIC}$, was observed to be 0.4%, 4.2%, and 1.2% for the +4, +3 and 2+ charge states respectively. For the same experiment, i between 225 V_{p-p} and 0 V_{p-p} RF voltage was measured to be 0.56 nA. The ion utilization efficiency, under these conditions, for angiotensin I was calculated to be $7.0 \pm 0.5\%$ by using eq. (4). In the same experiment, the EICs were summed for all observed peptide peaks in the mass spectrum and the fraction of analyte ions to the TIC, X_A , was calculated to be 0.43 with the theoretical maximum ion current, I_A , calculated to be 3.73 nA from eq. (3). This yields an overall ion utilization efficiency of $6.5 \pm 0.3\%$ for the single emitter/SPIN configuration by using eq. (5).

Table 1 shows the calculated ion utilization efficiencies for the individual peptides in the mixture as well as the overall efficiency under different interface configurations. The 1 μ M peptide mixture was used for all the measurements and calculations listed in Table 1. At a flow rate of 100 nL/min the overall ion utilization efficiency for the single emitter/single inlet ESI configuration was $4.0 \pm 0.3\%$, and increased only slightly to $4.2 \pm 0.8\%$ for a single emitter/multi inlet ESI configuration. Greater ion utilization efficiency was observed for the SPIN source with $6.4 \pm 0.3\%$ for single emitter/SPIN configuration and $13.0 \pm 2.3\%$ for the 10 emitter array/SPIN configuration even though the total flow rate is doubled (200 nL/min) in the 10 emitter array/SPIN configuration. In the 10 emitter array/SPIN

configuration, the flow is divided among the emitters and the effective flow rate at an individual emitter was ~10 fold lower than that of a single emitter at the same total liquid flow rate resulting in a much smaller charged droplets and thus more efficient ionization. To determine if the increase in ion utilization efficiency results directly from the decreased droplet size, the ion utilization efficiency of a single emitter in the SPIN source at a flow rate of 20 nL/min was determined. In this manner the flow rate at the emitter would be the same as the flow rate for an individual emitter in the emitter array. The calculated overall ion utilization efficiency was $26.7 \pm 1.8\%$, about two times higher than what was observed with the emitter array. The reduction in the overall ion utilization efficiency for the 10 emitter array/SPIN configuration as compared to the single emitter/SPIN configuration at the same flow rate per emitter is most likely due to the uneven flow division among the individual emitters in the array. The uneven flow division arises from slight emitter to emitter variation in the chemical etching procedure which will lead to nonuniform droplet sizes produced from individual emitters in the array lowering the efficiency. Alternatively, an emitter array operating at a flow rate of 200 nL/min will have a higher solvent flow than a single emitter operating at 20 nL/min which may cause decreased desolvation efficiency due to the larger solvent vapor pressure and cooling effects.

For the peptides examined in this study, the ion utilization efficiency differs significantly between peptides. For example the ion utilization efficiencies under the 10 emitter array/SPIN configuration for fibrinopeptide A, substance P, bradykinin, kemptide, and angiotensin II are similar in magnitude – ranging between 6% and 10% while the ion utilization efficiency for angiotensin I and neurotensin are much larger at $16.4 \pm 2.1\%$ and $34.5 \pm 3.6\%$ respectively. The ion utilization efficiency as high as over 50% can be achieved for neurotensin at ESI flow rate of 20 nL/min using the single emitter/SPIN configuration. Initially, ion suppression in the ESI process was considered to be responsible for the differences in ion utilization efficiencies among different peptides. However, further experimental measurements with the peptide mixture in the absence of neurotensin, the peptide with the highest ionization efficiency, showed that the ion utilization efficiencies remained essentially unaffected for all the remaining peptides. The results suggesting non-uniform ESI response of equal concentration analyte molecules regardless of ESI flow rates for peptides are consistent with a recent study with a conventional inlet capillary ESI configuration at ultra-low ESI flow rates [16].

CONCLUSIONS

To improve the sensitivity of mass spectrometric instrumentation it is important to understand and maximize the ion transmission at the ESI-MS interface. In this study we have studied and compared the ion transmission efficiencies of the conventional ESI-MS inlet capillary interface with the SPIN-MS interface. We found that the SPIN source transmits more than double the current through the first vacuum region of the instrument than the conventional ESI source which resulted in higher ion currents detected by the instrument. By calculating the theoretical maximum analyte ion current corresponding to all the analyte molecules in solution being completely converted to the gas phase ions and measuring gas phase ion current transmitted through the interface and the TIC and analyte EIC at the mass detector, it is possible to determine the ion utilization efficiency for any

given ESI-MS interface configurations. This established a metric to evaluate the overall efficiency of an ESI-MS interface design. Of the interfaces tested in this study, an emitter array/SPIN-MS interface demonstrated the greatest ion current, highest MS-signal intensity and subsequently the best ion utilization efficiency at a given total ESI flow rate.

Acknowledgments

We would like to thank Dr. Ryan T. Kelly and Dr. Yehia Ibrahim for useful discussions. Portions of this research were supported by the NIH National Cancer Institute (1R33CA155252) and General Medical Sciences (GM103493-12), the Laboratory Directed Research and Development Program at Pacific Northwest National Laboratory (PNNL), and the Department of Energy Office of Biological and Environmental Research Genome Sciences Program under the Pan-omics 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.

References

1. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray ionization for mass-spectrometry of large biomolecules. *Science*. 1989; 246:64–71. [PubMed: 2675315]
2. Smith RD, Loo JA, Loo RRO, Busman M, Udseth HR. Principles and practice of electrospray ionization - mass spectrometry for large polypeptides and proteins. *Mass Spectrometry Reviews*. 1991; 10:359–451.
3. Mehlis B, Kertscher U. Liquid chromatography mass spectrometry of peptides of biological samples. *Anal Chim Acta*. 1997; 352:71–83.
4. Shen Y, Toli N, Masselon C, Paša-Toli L, Camp DG, Hixson KK, Zhao R, Anderson GA, Smith RD. Ultrasensitive proteomics using high-efficiency on-line micro-spe-nanolc-nanoESI-MS and MS/MS. *Anal Chem*. 2003; 76:144–154. [PubMed: 14697044]
5. Wilm M, Mann M. Analytical properties of the nanoelectrospray ion source. *Anal Chem*. 1996; 68:1–8. [PubMed: 8779426]
6. El-Faramawy A, Siu KWM, Thomson BA. Efficiency of nano-electrospray ionization. *J Am Soc Mass Spectrom*. 2005; 16:1702–1707. [PubMed: 16095913]
7. Schmidt A, Karas M, Dulcks T. Effect of different solution flow rates on analyte ion signals in nano-ESI-MS, or: When does ESI turn into nano-ESI? *J Am Soc Mass Spectrom*. 2003; 14:492–500. [PubMed: 12745218]
8. Lin BW, Sunner J. Ion-transport by viscous-gas flow-through capillaries. *J Am Soc Mass Spectrom*. 1994; 5:873–885. [PubMed: 24226233]
9. Page JS, Kelly RT, Tang K, Smith RD. Ionization and transmission efficiency in an electrospray ionization-mass spectrometry interface. *J Am Soc Mass Spectrom*. 2007; 18:1582–1590. [PubMed: 17627841]
10. Kebarle P, Tang L. From ions in solution to ions in the gas-phase - the mechanism of electrospray mass-spectrometry. *Anal Chem*. 1993; 65:A972–A986.
11. Kebarle P. A brief overview of the present status of the mechanisms involved in electrospray mass spectrometry. *J Mass Spectrom*. 2000; 35:804–817. [PubMed: 10934434]
12. Bruins AP. Mechanistic aspects of electrospray ionization. *J Chromatogr A*. 1998; 794:345–357.
13. Konermann L, Ahadi E, Rodriguez AD, Vahidi S. Unraveling the mechanism of electrospray ionization. *Anal Chem*. 2012; 85:2–9. [PubMed: 23134552]
14. Marginean I, Kelly RT, Prior DC, LaMarche BL, Tang K, Smith RD. Analytical characterization of the electrospray ion source in the nanoflow regime. *Anal Chem*. 2008; 80:6573–6579. [PubMed: 18661954]
15. Heemskerk AAM, Busnel JM, Schoenmaker B, Derks RJE, Klychnikov O, Hensbergen PJ, Deelder AM, Mayboroda OA. Ultra-low flow electrospray ionization-mass spectrometry for improved ionization efficiency in phosphoproteomics. *Anal Chem*. 2012; 84:4552–4559. [PubMed: 22494114]

16. Marginean I, Tang K, Smith RD, Kelly RT. Picoelectrospray ionization mass spectrometry using narrow-bore chemically etched emitters. *J Am Soc Mass Spectrom.* 2014; 25:30–36. [PubMed: 24122304]
17. Tang KQ, Lin YH, Matson DW, Kim T, Smith RD. Generation of multiple electrosprays using microfabricated emitter arrays for improved mass spectrometric sensitivity. *Anal Chem.* 2001; 73:1658–1663. [PubMed: 11338576]
18. Su S, Gibson GTT, Mugo SM, Marecak DM, Oleschuk RD. Microstructured photonic fibers as multichannel electrospray emitters. *Anal Chem.* 2009; 81:7281–7287. [PubMed: 19715359]
19. Gibson GTT, Mugo SM, Oleschuk RD. Nanoelectrospray emitters: Trends and perspective. *Mass Spectrom Rev.* 2009; 28:918–936. [PubMed: 19479726]
20. Wu X, Oleschuk RD, Cann NM. Characterization of microstructured fibre emitters: In pursuit of improved nano electrospray ionization performance. *Analyst.* 2012; 137:4150–4161. [PubMed: 22706328]
21. Kelly RT, Page JS, Marginean I, Tang K, Smith RD. Nanoelectrospray emitter arrays providing interemitter electric field uniformity. *Anal Chem.* 2008; 80:5660–5665. [PubMed: 18553942]
22. Kim W, Guo M, Yang P, Wang D. Microfabricated monolithic multinozzle emitters for nanoelectrospray mass spectrometry. *Anal Chem.* 2007; 79:3703–3707. [PubMed: 17444614]
23. Mao P, Wang HT, Yang P, Wang D. Multinozzle emitter arrays for nanoelectrospray mass spectrometry. *Anal Chem.* 2011; 83:6082–6089. [PubMed: 21728281]
24. Schneider BB, Javaheri H, Covey TR. Ion sampling effects under conditions of total solvent consumption. *Rapid Commun Mass Spectrom.* 2006; 20:1538–1544. [PubMed: 16628560]
25. Manisali I, Chen DDY, Schneider BB. Electrospray ionization source geometry for mass spectrometry: Past, present, and future. *Trac-Trends in Analytical Chemistry.* 2006; 25:243–256.
26. Pagnotti VS, Inutan ED, Marshall DD, McEwen CN, Trimpin S. Inlet ionization: A new highly sensitive approach for liquid chromatography/mass spectrometry of small and large molecules. *Anal Chem.* 2011; 83:7591–7594. [PubMed: 21899326]
27. Wang B, Inutan E, Trimpin S. A new approach to high sensitivity liquid chromatography-mass spectrometry of peptides using nanoflow solvent assisted inlet ionization. *J Am Soc Mass Spectrom.* 2012; 23:442–445. [PubMed: 22238127]
28. Pagnotti VS, Chubaty ND, McEwen CN. Solvent assisted inlet ionization: An ultrasensitive new liquid introduction ionization method for mass spectrometry. *Anal Chem.* 2011; 83:3981–3985. [PubMed: 21528896]
29. Pagnotti VS, Chakrabarty S, Harron AF, McEwen CN. Increasing the sensitivity of liquid introduction mass spectrometry by combining electrospray ionization and solvent assisted inlet ionization. *Anal Chem.* 2012; 84:6828–6832. [PubMed: 22742705]
30. Page JS, Tang K, Kelly RT, Smith RD. Subambient pressure ionization with nanoelectrospray source and interface for improved sensitivity in mass spectrometry. *Anal Chem.* 2008; 80:1800–1805. [PubMed: 18237189]
31. Marginean I, Page JS, Tolmachev AV, Tang K, Smith RD. Achieving 50% ionization efficiency in subambient pressure ionization with nanoelectrospray. *Anal Chem.* 2010; 82:9344–9349. [PubMed: 21028835]
32. Pauly M, Sroka M, Reiss J, Rinke G, Albarghash A, Vogelgesang R, Hahne R, Kuster B, Sesterhenn J, Kern K, Rauschenbach S. A Hydrodynamically optimized nano-electrospray ionization source and vacuum interface. *Analyst.* 2014; 139:1856–1867. [PubMed: 24479126]
33. Cox JT, Marginean I, Kelly RT, Smith RD, Tang K. Improving the sensitivity of mass spectrometry by using a new sheath flow electrospray emitter array at subambient pressures. *J Am Soc Mass Spectrom.* 2014; 25:1007/s13361-014-0856-5
34. Kelly RT, Page JS, Luo Q, Moore RJ, Orton DJ, Tang K, Smith RD. Chemically etched open tubular and monolithic emitters for nanoelectrospray ionization mass spectrometry. *Anal Chem.* 2006; 78:7796–7801. [PubMed: 17105173]
35. Cech NB, Enke CG. Practical implications of some recent studies in electrospray ionization fundamentals. *Mass Spectrom Rev.* 2001; 20:362–387. [PubMed: 11997944]
36. Tang X, Bruce JE, Hill HH Jr. Characterizing electrospray ionization using atmospheric pressure ion mobility spectrometry. *Anal Chem.* 2006; 78:7751–7760. [PubMed: 17105168]

37. Tang KQ, Page JS, Smith RD. Charge competition and the linear dynamic range of detection in electrospray ionization mass spectrometry. *J Am Soc Mass Spectrom.* 2004; 15:1416–1423. [PubMed: 15465354]

Figure 1. Schematic of the dual ion funnel based SPIN-MS interface (a) and the heated inlet capillary ESI-MS interface (b).

Figure 2. Measured electric current transmitted through the high pressure ion funnel (a) and MS EIC (b) of the 3+ neurotensin ($m/z = 558.3$) for single emitter/single inlet ESI-MS (black squares), single emitter/multi-inlet ESI-MS (red diamonds), single emitter/SPIN-MS (blue triangles), and 10 emitter array/SPIN-MS interfaces (green circles) as a function of the high pressure ion funnel RF peak to peak voltage.

Figure 3.

(a) TIC with varying RF peak-to-peak voltages on the high pressure ion funnel and (b) representative mass spectra with the RF voltages of 225 V (black trace), and 0 V (red trace) using 100 nM peptide mixture for the single emitter/SPIN source configuration. The peptide peaks and their respective charge states have been annotated in the figure.

Figure 4.

Effect of liquid flow rate on Δi and analyte peak intensity for a single emitter/single inlet ESI-MS interface (a) and single emitter/SPIN interface (b). The red trace is Δi and the black, blue and green traces correspond to the EIC for 2+ angiotensin II ($m/z = 523.8$), 2+ bradykinin ($m/z = 530.8$), and 2+ fibrinopeptide A ($m/z = 768.9$) respectively.

Table 1

Comparison of ion utilization efficiencies (%) for different MS interface configurations.

Peptide	Single Emitter/Single Inlet ESI (100 nL/min)	Single Emitter/Multi-Inlet ESI (100 nL/min)	Single Emitter/SPIN (100 nL/min)	10 Emitter Array/SPIN (200 nL/min)	Single Emitter/SPIN (20 nL/min)
Fibrinopeptide A	4.8 ± 0.1	3.3 ± 0.6	9.6 ± 0.3	9.3 ± 0.7	17.2 ± 0.4
Substance P	3.9 ± 0.1	4.6 ± 1.1	6.0 ± 0.4	8.8 ± 1.6	34.6 ± 2.1
Angiotensinogen	4.9 ± 0.1	2.1 ± 0.6	5.6 ± 0.3	10.2 ± 2.0	11.0 ± 1.2
Neurotensin	3.4 ± 0.3	7.0 ± 1.2	12.9 ± 1.4	34.5 ± 3.6	50.3 ± 2.8
Bradykinin	3.9 ± 0.2	7.5 ± 1.1	2.8 ± 0.2	7.0 ± 1.6	30.0 ± 1.6
Angiotensin II	4.9 ± 0.2	5.1 ± 0.8	3.9 ± 0.7	9.6 ± 1.3	34.7 ± 1.8
Angiotensin I	5.1 ± 0.4	5.8 ± 1.2	7.0 ± 0.5	16.4 ± 2.1	48.7 ± 4.4
Kemptide	3.2 ± 0.1	5.0 ± 0.9	1.9 ± 0.4	6.0 ± 0.5	20.7 ± 1.1
Overall Ion utilization efficiency	4.0 ± 0.3	4.2 ± 0.8	6.5 ± 0.3	13.0 ± 2.3	26.7 ± 1.8