

Characterization of Charge Separation in the Array of Micromachined UltraSonic Electrospray (AMUSE) Ion Source for Mass Spectrometry

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An initial investigation into the effects of charge separation in the Array of Micromachined UltraSonic Electrospray (AMUSE) ion source is reported to gain understanding of ionization mechanisms and to improve analyte ionization efficiency and operation stability. In RF-only mode, AMUSE ejects, on average, an equal number of slightly positive and slightly negative charged droplets due to random charge fluctuations, providing inefficient analyte ionization. Charge separation at the nozzle orifice is achieved by the application of an external electric field. By bringing the counter electrode close to the nozzle array, strong electric fields can be applied at relatively low DC potentials. It has been demonstrated, through a number of electrode/electrical potential configurations, that increasing charge separation leads to improvement in signal abundance, signal-to-noise ratio, and signal stability. (J Am Soc Mass Spectrom 2009, 20, 1684–1687) © 2009 American Society for Mass Spectrometry

Electrospray ionization (ESI) allows the generation of intact, low internal energy, gas-phase ions from molecules in solution through the use of electrohydrodynamic focusing of an applied electric field, known as a Taylor cone [1–5]. There is a strong momentum to develop miniaturized sample introduction platforms and lab-on-a-chip ion sources for proteomics because samples available for analysis are frequently difficult to obtain and small in volume. Microfabricated nanoelectrospray devices enable enhanced sensitivity and multiplexed mass spectrometric (MS) analysis with improved reproducibility as well as provide a natural path to coupling with on-chip micro-fabricated separation columns [6, 7]. Although many of these devices lead to enhancements in some aspects of gas-phase ion production, they still rely on highly charged capillaries to generate Taylor cone electrospray and thus suffer from its limitations. A novel ion source, Array of Micromachined UltraSonic Electrospray (AMUSE), conceptually differs from classical electrospray ionization (ESI) by independently controlling analyte charging and droplet/ion formation processes [8–12], thus allowing for low voltage ultra-soft ionization of a variety of analytes and flexibility in the choice of solvents [13–15]. Most recently,

multiplexed operation of the AMUSE has been demonstrated, thus establishing a direct path to a multiplexed ion source for mass spectrometry [12]. Additionally, Hampton et al. [15] compared side-by-side the internal energy deposition between conventional ESI and AMUSE to conclusively show that a superior softness of analyte ionization can be achieved by AMUSE under certain operating conditions.

In the case of ESI, the large electric field causes charge separation, which forms a Taylor cone that ejects charged droplets when the repulsion force overcomes surface tension [16]. One of the main benefits of the AMUSE ion source is its ability to separate droplet formation from charge separation. When operating the AMUSE in RF-only mode, without any external electric field, on average, an equal number of slightly positive and slightly negative charged droplets are ejected due to random charge fluctuations. While this is sufficient to achieve analyte ionization at higher analyte concentrations, such a mode of operation is not optimal [14]. To maximize the net charge placed on individual droplets upon ejection and hence improve ionization efficiency and sensitivity, charge separation must be achieved. In previous studies, it has been shown that a DC-potential-biased external electrode could be successfully used to polarize a neutral spray from a pneumatic nebulizer [17, 18]. When a similar idea is applied to the AMUSE ion source, the induced electric field can force the positive charges (positive

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mode of operation) toward the fluid-air interface where a droplet is formed, while the negative charges will be left in the bulk solution. The electrochemical oxidation of relevant anions at the piezoelectric transducer's top electrode facing the solution neutralize these negative charges, similarly to the ESI, resulting in stable device operation as a closed circuit electrochemical cell. Under these conditions, droplets with a much greater net (positive) charge are ejected resulting in an improved ionization efficiency, stability, and sensitivity of MS detection. An effect of applying a DC bias to the piezoelectric transducer electrode was initially considered in our earlier work [13, 14], but only briefly and lacking an in-depth look into a physical mechanism responsible for improved ionization. An initiation of the latter is the focus of the work reported here.

Experimental

Materials

Reserpine, purchased from Sigma-Aldrich (St. Louis, MO, USA) was used as received. Micromolar solutions of the compound were prepared in deionized water (Ricca Chemical Company, Arlington, TX, USA) containing 0.1% (vol/vol) glacial acetic acid (BDH Aristar, Westchester, PA, USA). No organic solvents were used in the working solutions.

AMUSE Ion Source

The AMUSE ion source fabrication and assembly have been described in detail elsewhere [9, 14]. Compared with the standard assembly, new to this investigation is addition of a brass support, containing a transformer wire (electrically shielded) electrode. As seen in Figure 1 (insert), this electrode acts as a "counter" electrode in the charge separation experiments. The experiments are completed for a 3 μ M solution of reserpine delivered to

the AMUSE fluid reservoir at 30 to 50 μ L/min using a syringe pump. Bias DC potentials are applied to the inner electrode of the piezoelectric transducer, V_{PZT} , and the external wire counter electrode, V_{ext} , allowing a precise control of the induced electric field strength.

Air Amplifier

For mass spectrometry operation, the AMUSE ion source is coupled in-line with an air amplifier (EXAIR Corporation, Cincinnati, OH, USA) to improve collection and transport of droplets/ions to the mass spectrometer inlet (Figure 1). The air amplifier is also used to assist in droplet desolvation, by heating the assisting nitrogen gas flow with a coil heater (Omega, Stamford, CT, USA). Here, the AMUSE is set in an orthogonal orientation to the assisting air flow for visual inspection of device operation under various conditions.

MicroTOF Mass Spectrometer

The AMUSE source was coupled to a time-of-flight mass spectrometer (Bruker Daltonics microTOF, Billerica, MA, USA). All experiments were conducted in positive ionization mode with the air amplifier, V_{AA} , and mass spectrometer inlet, V_{MS} , grounded (zero DC potential relative to a common electric ground). The TOF capillary was maintained at 180 °C with a dry gas counter-flow rate of 3 L/min and spectra collected is a rolling average at a scan rate of 3 Hz.

Data Analysis

All mass spectra obtained are collected for a 1 min acquisition period. Reported data points are the average of 3 to 8 acquisition period datasets, collected under the same conditions on different days. The reported signal intensities are the maximum intensity found from an extracted ion chromatogram of the base peak.

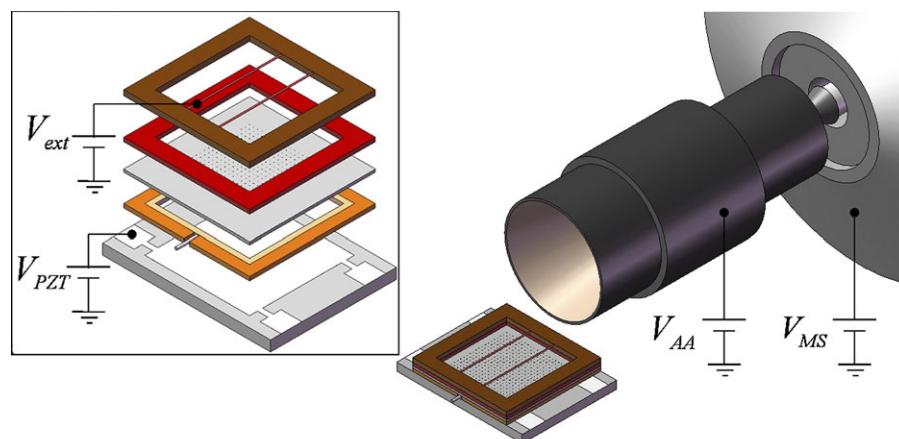


Figure 1. Schematic of the experimental system coupling AMUSE to air amplifier to time-of-flight mass spectrometer, including the electrode placement and locations with controlled DC electric bias potentials. The insert provides an exploded view of the AMUSE ion source components.

Results and Discussion

A methodical investigation into the effect of electrical field configuration and strength on the signal intensity and stability has been completed with the use of a micrOTOF mass spectrometer (Figure 1). Two crossing wires are used as a counter electrode providing an electric field in the direction normal to the nozzle array, while minimizing ejection blockage due to the wires. MS intensity values are taken from the extracted ion chromatogram as shown in Figure 2 (top) and a representative mass spectrum (bottom) for reserpine.

Figure 3 shows the maximum signal intensity of the base peak as a function of the DC electric potential difference applied between the electrodes (i.e., the piezoelectric transducer and the wire) across the device. In the first dataset (squares) a positive DC potential is applied to the piezoelectric electrode, $V_{PZT} = +V_{DC}$, and the wire counter electrode is grounded, $V_{ext} = 0$. The resulting electric field induces charge separation by moving positive charges toward the nozzle orifice. As the data shows, increasing the potential drop, $\Delta\phi_{\square} = V_{PZT} - V_{ext}$, increases the signal intensity and improves signal-to-noise ratio (S/N) from ~60 up to values greater than 2000. An order of magnitude increase in the electric field strength results in two orders of magnitude increase in S/N ratio. The second dataset (diamonds) is for the case when the wire counter electrode is removed, while leaving the remainder of the setup the same. The relevant potential difference that defines the electric field strength now becomes $\Delta\phi_{\diamond} = V_{PZT} - V_{AA}$. This configuration is similar to that used in the initial characterization and validation of the AMUSE ion source [13–15]. In this configuration, the

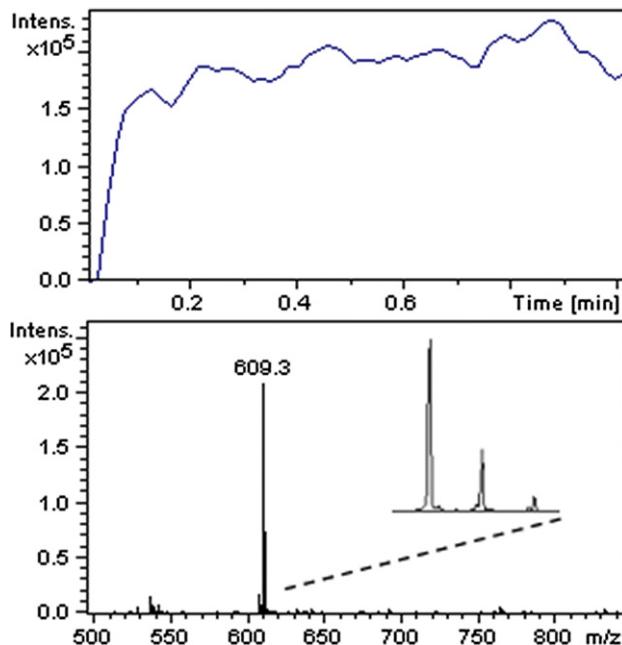


Figure 2. Extracted ion chromatogram of a base peak (top), and representative mass spectrum (bottom), for 3 μ M reserpine in 99.9:0.1 (vol/vol) water/acetic acid ionized by the AMUSE.

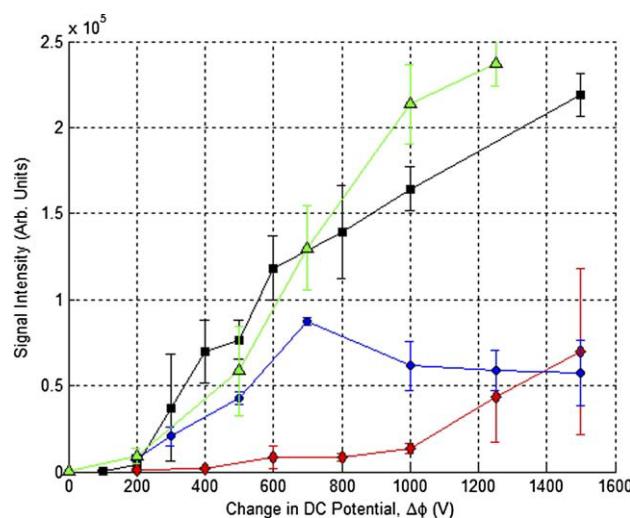


Figure 3. Maximum signal intensity obtained with the AMUSE ion source for 3 μ M reserpine in 99.9:0.1 (vol/vol) water/acetic acid for various applied electric field configurations. Potential configurations: case 1 (squares): ($V_{PZT} = +V_{DC}$, $V_{ext} = 0$), case 2 (diamonds): ($V_{PZT} = +V_{DC}$, $V_{ext} = \text{removed}$), case 3 (circles): ($V_{PZT} = 0$, $V_{ext} = -V_{DC}$), case 4 (triangles): ($V_{PZT} = +V_{DC} = \text{Const}$, $V_{ext} = +V_{DC}$), and for all cases, $V_{AA} = V_{MS} = 0$. For cases 1 (squares), 3 (circles), and 4 (triangles): $\Delta\phi = V_{PZT} - V_{ext}$ and for case 2 (diamonds): $\Delta\phi = V_{PZT} - V_{AA}$.

air amplifier acts as the counter electrode, and moving it further away results in a decreased electric field. This, in turn, yields a reduced charge separation. As the potential is increased, $V_{PZT} > 1000$ V, the electric field eventually becomes sufficiently strong to support noticeable charge separation at the point of droplet ejection and the signal intensity experiences a modest gain. It is apparent and hardly surprising that the electric field strength, and not just the potential drop, is determining the charge separation and therefore the extent of the signal improvement.

The third dataset (circles) describes experiments in which all applied DC potentials were altered, relative to a common electric ground, but proportionally; therefore, no effect is expected on the strength of the electric field. In particular, the piezoelectric electrode was grounded, $V_{PZT} = 0$, and a negative DC bias was applied to the wire electrode, $V_{ext} = -V_{DC}$, with potential difference defined as $\Delta\phi_o = V_{PZT} - V_{ext}$. This configuration was intended to isolate the signal improvement due to charge separation by eliminating any signal increase/decrease that may be due to increased/decreased charge levels due to electrochemical processes at the piezoelectric transducer electrode. As shown in Figure 3, the signal intensity begins to increase with an increase in the applied electric field in line with the measurements obtained for the first dataset. However, upon reaching a certain potential difference, the signal intensity begins to drop with further increase in the potential difference. The source for discrepancy between the first (squares) and third (circles) datasets at higher $\Delta\phi_o = V_{PZT} - V_{ext}$ becomes apparent if one considers not only the magnitude, but

also the direction of the electric field along the entire ion/charged droplet transmission path from the ejection point to the mass spectrometer inlet (**Figure 1**). In the first (squares) set of experiments, the wires were grounded so no external electric field existed between the wire electrode and air amplifier. In contrast, for the third dataset (circles), although the local electric field between the AMUSE ejection surface and the wire electrode is the same, there is now an adverse electric field between the negatively biased wires ($V_{ext} = -V_{DC}$) and a grounded air amplifier ($V_{AA} = 0$) that hinders positive ion transport to the MS inlet. Thus, while the charge separation remains equally effective in the latter (third) set of experiments, the measured MS signal intensity decreases at higher piezo-to-wire potential differences due to diminishing the charged droplet/ion transmission between the wire electrode and the air amplifier/MS inlet when the droplets contain high levels of charge. This conclusion is further supported by the fact that while the signal intensity is depressed at larger $\Delta\phi_o = V_{PZT} - V_{ext}$ operation, the S/N ratio does not, maintaining ~ 1500 . To summarize, while charge separation helps increase the charge density in ejected droplets, ion transport remains a vital process in MS operation with the AMUSE ion source.

The final dataset (triangles) further aims to eliminate the effects of solvent oxidation by applying a constant (positive) potential to the piezoelectric electrode, $V_{PZT} = +V_{DC} = Const$, while positively biasing the wire electrode, $V_{ext} = +V_{DC}$, ($\Delta\phi_\Delta = V_{PZT} - V_{ext}$). Again, the electric field across the AMUSE is the same as for the first case and, as expected, the data follows each other very closely. The slight increase in an MS signal at high potential difference can be attributed to the small ion-transport-assisting electric field produced between the wire electrodes and air amplifier. The experimental data from these tests clearly show that an increase in the external electric field strength at the point of droplet ejection improves the signal intensity and S/N ratio, as long as the electric field distribution is optimized between the ion source and the mass spectrometer inlet. We also find a direct correlation between increasing the local electric field and experiment repeatability.

Conclusions

MS characterization of various electrode configurations in the AMUSE ion source indicates that an external electric field localized and focused at the ejection interface near the nozzle orifice induces efficient charge separation, resulting in the ejection of increasingly charged droplets. An increased charge density within droplets improves both the signal-to-noise ratio (sensitivity) and signal stability, allowing for more efficient analyte ionization. An in-depth computational analysis of charge separation, including electrohydrodynamic considerations during the droplet ejection in the presence of an electric field, will help understand the physics of charge separation and

quantify charge deposited on individual droplets, while developing an insight into droplet evolution in the presence of an electric field. Such a computational analysis, which is the focus of the follow-up article to this communication, will not only improve fundamental understanding, but will also be instrumental to an improved design and optimal operation of the AMUSE-type droplet-based ion sources.

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References

- Dole, M.; Mack, L. L.; Hines, R. L.; Mobley, R. C.; Ferguson, L. D.; Alice, M. B. Molecular Beams of Macroions. *J. Chem. Phys.* **1968**, *49*(5), 2240–2249.
- Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. Electrospray Ionization for Mass Spectrometry of Large Biomolecules. *Science* **1989**, *246*(4926), 64–71.
- Gaskell, S. J. Electrospray: Principles and Practice. *J. Mass Spectrom.* **1997**, *32*(7), 677–688.
- Cech, N. B.; Enke, C. G. Practical Implications of Some Recent Studies in Electrospray Ionization Fundamentals. *Mass Spectrom. Rev.* **2001**, *20*(6), 362–387.
- Gabelica, V.; Pauw, E. D. Internal Energy and Fragmentation of Ions Produced in Electrospray Sources. *Mass Spectrom. Rev.* **2005**, *24*(4), 566–587.
- Lazar, I. M.; Grym, J.; Foret, F. Microfabricated Devices: A New Sample Introduction Approach to Mass Spectrometry. *Mass Spectrom. Rev.* **2006**, *25*(4), 573–594.
- Limbach, P. A.; Meng, Z. Integrating Micromachined Devices with Modern Mass Spectrometry. *Analyst* **2002**, *127*(6), 693–700.
- Fedorov, A. G.; Degertekin, F. L. *Electrospray Systems and Methods* U.S. Patent no. 7,208,727.
- Meacham, J. M. A Micromachined Ultrasonic Droplet Generator: Design, Fabrication, Visualization, and Modeling. Doctoral Dissertation, Georgia Institute of Technology, 2006.
- Meacham, J. M.; Ejimofor, C.; Kumar, S.; Degertekin, F. L.; Fedorov, A. G. Micromachined Ultrasonic Droplet Generator Based on a Liquid Horn Structure. *Rev. Sci. Instrum.* **2004**, *75*(5), 1347–1352.
- Meacham, J. M.; Varady, M. J.; Degertekin, F. L.; Fedorov, A. G. Droplet Formation and Ejection from a Micromachined Ultrasonic Droplet Generator: Visualization and Scaling. *Phys. Fluids* **2005**, *17*(10), 100605–100608.
- Forbes, T. P.; Degertekin, F. L.; Fedorov, A. G. Multiplexed Operation of a Micromachined Ultrasonic Droplet Ejector Array. *Rev. Sci. Instrum.* **2007**, *78*(10), 104101–104106.
- Aderogba, S.; Meacham, J. M.; Degertekin, F. L.; Fedorov, A. G.; Fernandez, F. M. Nanoelectrospray Ion Generation for High-Throughput Mass Spectrometry Using a Micromachined Ultrasonic Ejector Array. *Appl. Phys. Lett.* **2005**, *86*(20), 203110–203113.
- Hampton, C. Y.; Forbes, T. P.; Varady, M. J.; Meacham, J. M.; Fedorov, A. G.; Degertekin, F. L.; Fernandez, F. M. Analytical Performance of a Venturi-Assisted Array of Micromachined Ultrasonic Electrosprays Coupled to Ion Trap Mass Spectrometry for the Analysis of Peptides and Proteins. *Anal. Chem.* **2007**, *79*(21), 8154–8161.
- Hampton, C. Y.; Silvestri, C. J.; Forbes, T. P.; Varady, M. J.; Meacham, J. M.; Fedorov, A. G.; Degertekin, F. L.; Fernandez, F. M. Comparison of the Internal Energy Deposition of Venturi-Assisted Electrospray Ionization and a Venturi-Assisted Array of Micromachined UltraSonic Electrosprays (AMUSE). *J. Am. Soc. Mass Spectrom.* **2008**, *19*(9), 1320–1329.
- Fernandez de la Mora, J.; Van Berkel, G. J.; Enke, C. G.; Cole, R. B.; Martinez-Sanchez, M.; Fenn, J. B. Electrochemical Processes in Electrospray Ionization Mass Spectrometry. *J. Mass Spectrom.* **2000**, *33*(8), 939–952.
- Thomson, B. A.; Iribarne, J. V. Field Induced Ion Evaporation from Liquid Surfaces at Atmospheric Pressure. *J. Chem. Phys.* **1979**, *71*(11), 4451–4463.
- Iribarne, J. V.; Dziedzic, P. J.; Thomson, B. A. Atmospheric Pressure Ion Evaporation-Mass Spectrometry. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *50*(3), 331–347.