

**Final report of activities carried out at the  
Georgia Institute of Technology Supported  
by ASMS Research award.**

**Period: February 2005-May 2007**

**To: American Society for Mass Spectrometry**  
**From: Prof. Facundo M. Fernandez (School of Chemistry and Biochemistry, Georgia Institute of Technology)**

**Mayor Activities carried out under support by the ASMS Research Award.**

During the period of February 2005 to May 2007 we have actively characterized, optimized, and further developed the array of acoustic micromachined ultrasonic electrosprays (AMUSE) first invented by our collaborators Fedorov and Degertekin (Electrospray Systems and Methods, Patent 7,208,727, issued 04/24/2007), and described in the initial proposal. Leveraging the support from the ASMS research award, federal funding was secured via a three year grant from NIH (R21). The main activities carried out at the Georgia Institute of Technology in the Fernandez group during the period covered by this report are summarized below:

*Mass Spectrometry Interfacing Effort:*

Initial experiments in our labs were aimed at coupling the AMUSE device to QqQ and TOF mass spectrometers using a direct (non-Venturi) interface, as we described earlier (*App. Phys. Lett.*, **2005**, 86, 203110/1-203110/3). These experiments were only marginally successful, i.e. although spectra could be obtained, sensitivity was low. Most importantly, stability was limited to only a few seconds of stable operation in combination with the mass spectrometer. This effect was attributed to the poor droplet collection efficiency at the micron-sized orifice inlets of these two spectrometers (API III, and AccuTOF Jeol). In addition, these two instruments do not make use of a heated capillary at the initial stage of the API interface, which could explain the poor ion yield and stability observed.

In order to mitigate these problems, two modifications to our initial setup were conducted. First, experiments were carried out with an ion trap instrument, which enables to trap ions for extended periods of time, thus mitigating temporal variations in the ion stream. Secondly, a Venturi interface was developed, tested and characterized. Several AMUSE device assembly methods were assayed in terms of stability, tolerance to leakage and ejection efficiency. A “multilayer” type assembly developed by our collaborators at the School of Mechanical Engineering (Degertekin Group) seemed to produce the best results.

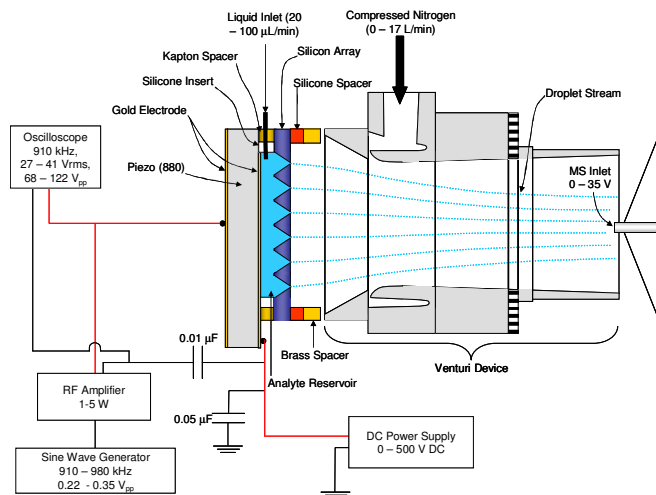
Based on an assembly of the AMUSE device developed by the Degertekin group we assembled a Venturi-based MS interface. Figure 1 shows the Venturi-assisted AMUSE-MS setup developed by the Fernandez group. In this setup, lead wires were soldered onto the inner and outer gold electrodes and were connected to a DC power supply (PS325, Stanford Research Systems, Inc., Sunnyvale, CA) and RF amplifier (Ultra 2020, T&C Power Conversion, Rochester NY), respectively. Two types of devices were used for most experiments. First generation devices with 5 $\mu$ m nozzles, and membrane-coated 3  $\mu$ m devices. For 5  $\mu$ m devices, a 980 kHz sine wave with a 0.220 Vpp amplitude produced by a function generator (DS345, Stanford Research Systems, Inc., Sunnyvale, CA) was used to drive the piezo transducer. For 3  $\mu$ m devices, a 910 kHz sine wave, with a 0.300 VPP amplitude was used. The amplified RF potential was directly applied to the outer gold electrode causing periodic deformation of the piezoelectric transducer. A medium voltage power supply was directly connected to the inner gold electrode on the piezoelectric transducer, and used to apply charging voltages of 0-500 V<sub>DC</sub> to the analyte-containing solution. The fully assembled AMUSE device was placed approximately 2 mm from the inlet of a stainless steel Venturi device (SS10A Airmover, HMC Brauer Co., Milton Keynes, England), operated at a rate of 0-9.5 L min<sup>-1</sup> nitrogen at room temperature. Nitrogen flow rate was controlled using a precision flow meter. For experiments with proteins, the nitrogen was heated using an inline air circulation heater (Omega) and the temperature was controlled using a temperature controller (Eppendorf, TC-50). The Venturi device increased the volumetric flow

rate of the exiting air stream to approximately  $40 \text{ L min}^{-1}$ , thus improving the desolvation efficiency, and reducing the droplet size. Most importantly, it improved the droplet linear velocity, thus improving the efficiency of droplet transmission into the mass spectrometer.

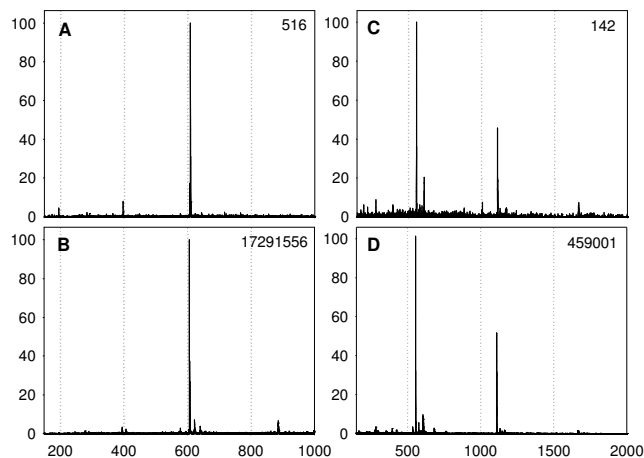
Initial experiments with first generation AMUSE devices ( $5 \text{ }\mu\text{m}$  nozzles) were performed with a linear ion trap (LiT) mass spectrometer (LTQ, ThermoFinnigan, Waltham, MA). Experiments with second generation devices were performed with a quadrupolar ion trap (QiT) mass spectrometer (LCQ Deca XP+, ThermoFinnigan, Waltham, MA). In both cases, the standard electrospray ion source was removed, and the Venturi device was positioned directly in front of the heated capillary inlet. Extensive characterization studies were performed with this interface, which are being prepared for publication. The main achievement in this period is the successful soft ionization via AMUSE of peptides, drug molecules and proteins from acidified water solutions in the absence of organic solvents. Figure 2 shows representative reserpine and Leu-Enk spectra obtained with different-generation AMUSE devices. As mentioned before, these spectra were obtained in the absence of organic solvents, i.e. from acidified aqueous solutions, thus making this approach more directly applicable to the study of biomolecules in states closer to their native conformations.

#### *Electrodynamic Characterization of AMUSE-MS*

The sensitivity and stability of the Venturi-assisted AMUSE device were characterized as a function of several variables such as air amplifier gas flow rate, ion collection distances and DC charging potentials. Possibly, the most interesting results were observed for the effect of the charging potential, which are presented here (Figure 3). It was observed that successful ionization of model peptides was possible with voltages as low as  $50 \text{ V DC}$ , with a maximum in SNR observed at  $\sim 300 \text{ V DC}$ . Surprisingly, we also observed successful charging and ion generation at null DC voltages. Possibly, the residual charge accumulated on the ejected droplets during time-



**Figure 1: Venturi-assisted AMUSE-MS setup.**



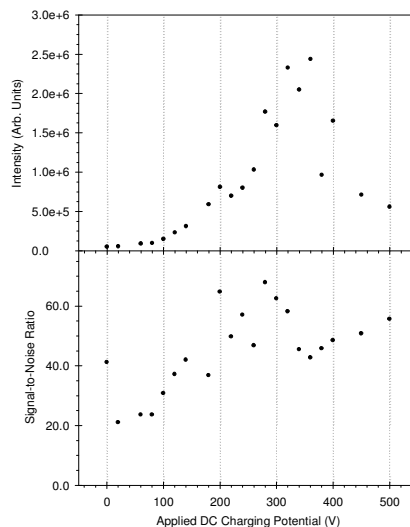
**Figure 2: Mass spectra produced by averaging 1.2 sec of data obtained with different devices. (A) reserpine on a  $5 \text{ }\mu\text{m}$  chip, (B) reserpine on a  $3 \text{ }\mu\text{m}$  chip, (C) leucine enkephalin on a  $5 \text{ }\mu\text{m}$  chip, and (D) leucine enkephalin on a  $3 \text{ }\mu\text{m}$  chip. All solutions were prepared using  $99.9:0.1 \text{ (v:v)}$  water:glacial acetic acid as the solvent. Operating conditions: (A) and (C):  $100 \text{ V}_{\text{DC}}$ ,  $100 \text{ }\mu\text{L/min}$  liquid flow rate,  $7.5 \text{ L/min N}_2$ , (B) and (D):  $100 \text{ V}_{\text{DC}}$ ,  $30 \text{ }\mu\text{L/min}$  liquid flow rate,  $9.3 \text{ L/min N}_2$ .**

dependent pressure gradient generated at the cavity apex is sufficient to incorporate sufficient charge on the ejected liquid to produce a detectable amount of protonated molecule ions. Operation in this mode completely avoids the need of a separate power supply for DC charging, thus opening an avenue for more compact, and simpler instrumentation.

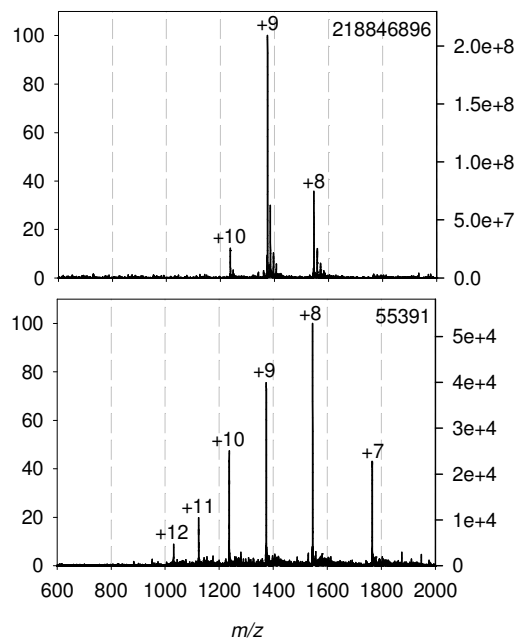
### AMUSE-TOF for medium complexity bio-samples

As a first step in the characterization of complex biological molecules with AMUSE, model protein samples were studied. Figure 4 shows standard nanospray (NSI) spectra and AMUSE spectra of the model protein cytochrome C. Successful protein ionization was observed when the air amplifier gas stream was heated to 100 °C. This is the first successful example of protein ionization with this device, serving as a proof-of-principle of the usefulness of this approach for biological mass spectrometry.

It can be observed in Figure 4 that AMUSE and standard NSI produced spectra with different charge distributions. It was observed that AMUSE produced a higher abundance of more compact protein charge states (i.e. more native-like), and a wider distribution of conformers. As the ionization mechanisms in NSI and AMUSE are very different, there is not a single variable that can explain the observed differences. Differences in average droplet size, charges, and local droplet pH between the two devices can explain the observed differences in charge states. Further studies at varying solution pH are necessary to fully understand the observed differences, but the results presented in Figure 4 demonstrate that protein ionization with good sensitivity is possible with the AMUSE device. We also believe that once we better understand the factors governing the internal energy deposition of this type of ion source, we will be able to narrow down the charge state distribution and further preserve solution phase conformation. Although the observed sensitivity for AMUSE was good, at this point the observed SNR is still lower than for NSI, despite the fact that 3 times more protein was ejected per nozzle in the case of AMUSE. We expect that further improvement in the ion transmission interface, such as positioning the inlet capillary at the interior of the Venturi device, will help to overcome these differences in sensitivity.



**Figure 3: Representative MS signal (a.u.) and signal-to-noise ratio as function of the DC bias voltage in AMUSE-MS experiments model peptides**



**Figure 4 Mass spectra of cytochrome C in 99.9:0.1 (v:v) water:acetic acid obtained using (A) standard nanospray ionization (0.8 pmol; 0.8  $\mu$ L/min; 2500 V<sub>DC</sub>), (B) AMUSE ionization using a chip with membrane-coated 3  $\mu$ m nozzles (240 pmol total, ~2.4 pmol/nozzle, 60  $\mu$ L/min liquid flow rate; 100 V<sub>DC</sub>; 8 L/min N<sub>2</sub> heated to 100 °C. QiT detection).**

## SIGNIFICANCE

The findings described in this report have a central significance for the development of the AMUSE device into a powerful ion source for proteomics and metabolomics. We have, for the first time, demonstrated the successful ionization of biological molecules with this source and we are now preparing these results for publication. We have also started work to further characterize the internal energy deposition during the AMUSE ionization process. This involves extended chemical synthesis of a series of 9 different p-substituted aminopyridines (thermometer molecules).

## ACHIEVED MILESTONES & FUTURE PLANS

We have achieved stable AMUSE MS operation for periods as long as 50 minutes, using acidified water as the base solvent. Spectra for as low as 10amol/nozzle for reserpine, 200fmol/nozzle for CytC, and 100fmol for YGGFL have been obtained. The next hurdles to be overcome involve the increase of the number of operating nozzles (at this point we achieve approximately between 25 and 50% of all nozzles ejecting at the same time, probably due to incomplete filling of the reservoir cavity, or clogging of the remaining nozzles. We have observed that stability can be seriously affected by liquid buildup on the device surface. We have now received parylene-coated devices from the Fedorov and Degertekin groups, which we plan to test shortly in our Venturi setup. We have also observed that ejection in the absence of DC charging voltages does not result in such significant liquid buildup, thus hinting that this phenomenon is driven by the surface chemistry at the nozzle boundaries.

## PUBLICATIONS AND PRESENTATIONS

*Article in preparation.*

- “Venturi-assisted Ion Generation by an Array of Micromachined Ultrasonic Electrosprays (AMUSE) for Linear and Quadrupolar Ion Trap Mass Spectrometry”, Christina Y. Hampton, Thomas P. Forbes, Mark J. Varady, J. Mark Meacham, Andrei G. Fedorov, F. Levent Degertekin, Facundo M. Fernandez.

*Oral Presentation at conferences. (presenting author underlined):*

- “Characterization of Ion Generation by a Venturi-assisted Array of Micromachined Ultrasonic Electrosprays”, Facundo M. Fernandez, Christina Y. Hampton, Thomas P. Forbes, J. Mark Meacham, Robert B. Dixon, Catherine Silvestri, David C. Muddiman, F. Levent Degertekin, Andrei G. Fedorov, 2007 ASMS Conference, June 3-7, 2007, Indianapolis, IN.
- “Venturi-assisted Nanospray Protein Ion Generation by a Micromachined Ultrasonic Electrospray Array”, Facundo M. Fernandez, Christina Y. Hampton, Mark Meacham, Levent Degertekin, Andrei Fedorov, 17<sup>th</sup> International Mass Spectrometry Conference, Prague, Czech Republic, Aug. 27-Sept 1<sup>st</sup>, 2006.

*Other Oral presentations:*

- “Venturi-assisted Ion Generation by a Micromachined Array of Ultrasonic Electrosprays”, Christina Y. Hampton, Thomas P. Forbes, Mark J. Varady, J. Mark Meacham, Catherine J. Silvestri, F. Levent Degertekin, Andrei G. Fedorov, Facundo M. Fernandez 2007 AAMSDG Symposium, April 25-26<sup>th</sup>, 2007, University of Georgia, Athens, GA.
- "From High Vacuum to Open Air: Frontiers in Ionization Methods for MS-based Forensics and Proteomics", Facundo Fernandez, Minnesota Mass Spectrometry Discussion Group (MinnMass), February 10<sup>th</sup>, 2007. Host: Peter Vlasak.

- "Vacuum is not the Limit: High-Throughput Mass Spectrometry in the Open Air", Facundo Fernandez, Athens-Atlanta Mass Spectrometry Discussion Group (AAMSDG), December 4<sup>th</sup>, 2006. Host: Ron Orlando.
- "Vacuum is not the Limit: Enabling Mass Spectrometry Technologies for High-Throughput Proteomics and Metabolomics in the Open Air", Facundo Fernandez, November 1<sup>st</sup>, 2006, Center for Environmental Systems Microbiology Research Forum, Georgia Institute of Technology, November 1<sup>st</sup>, 2006. Host: Patty Sobecky.

*Poster presentations:*

- "AMUSE (Array of Micromachined UltraSonic Electrospray) Ion Source for Bioanalytical Mass Spectrometry", Thomas P. Forbes, F. Levent Degertekin, Christina Y. Hampton, J. Mark Meacham, Facundo M. Fernandez, R. Brent Dixon, David C. Muddiman, and Andrei G. Fedorov. Integrated Biosystems Initiative Poster Session, March 14, 2007. Georgia Institute of Technology, Atlanta, GA.
- "Mass Spectrometric Characterization of the Performance of a Venturi-assisted Array of Micromachined Ultrasonic Electrosprays (AMUSE)", Christina Y. Hampton, Thomas P. Forbes, J. Mark Meacham, Robert B. Dixon, David C. Muddiman, F. Levent Degertekin, Andrei G. Fedorov, and Facundo M. Fernandez, Integrated Biosystems Initiative Poster Session, March 14, 2007. Georgia Institute of Technology, Atlanta, GA.
- "AMUSE (Array of Micromachined UltraSonic Electrospray) Ion Source for High Throughput, Multiplexed Bioanalytical Mass Spectrometry" Thomas P. Forbes, Christina Y. Hampton, J. Mark Meacham, Facundo M. Fernandez, F. Levent Degertekin, R. Brent Dixon, David C. Muddiman, and Andrei G. Fedorov, Third HUPO Annual Conference, March 5-8, 2007, Seattle, WA.
- "Protein Ion Generation by a Venturi-assisted Micromachined Ultrasonic Electrospray Array", Christina Y. Hampton, J. Mark Meacham, Facundo M. Fernandez, Andrei G. Fedorov and F. Levent Degertekin. 58<sup>th</sup> Southeastern Regional Meeting of the ACS. November 1-4, 2006. Augusta, GA.