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Increasing the Sensitivity of Liquid Introduction Mass Spectrometry by Combining Electrospray Ionization and Solvent Assisted Inlet Ionization

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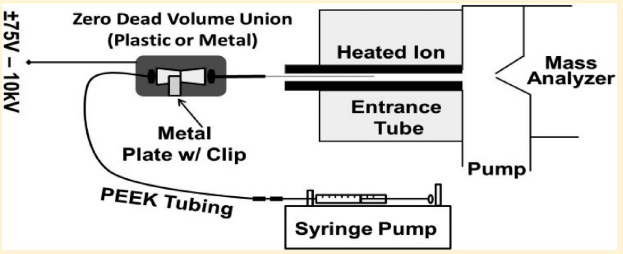
ABSTRACT: Combining electrospray ionization (ESI) and solvent assisted inlet ionization (SAII) provides higher ion abundances over a wide range of concentrations for peptides and proteins than either ESI or SAII. In this method, a voltage is applied to a union connector linking tubing from a solvent delivery device and the fused silica capillary, used with SAII, inserted into a heated inlet tube of an Orbitrap Exactive mass spectrometer (MS). The union can be metal or polymeric and the voltage can be applied directly or contactless. Solution flow rates from less than a 1 μL min^{-1} to over 100 μL min^{-1} can be accommodated. It appears that the voltage is only necessary to provide charge separation in solution, and the hot MS inlet tube and the high velocity of gas through the tube linking atmospheric pressure and vacuum provides droplet formation. As little as 100 V produces an increase in ion abundance for certain compounds using this method relative to no voltage. Interestingly, the total ion current observed with SAII and this electrosprayed inlet ionization (ESII) method are very similar for weak acid solutions, but with voltage on, the ion abundance for peptides and proteins increase as much as 100-fold relative to other compounds in the solution being analyzed. Thus, switching between SAII (voltage off) and ESII (voltage on) provides a more complete picture of the solution contents than either method alone.



Numerous approaches to ionization for mass spectrometry (MS) have been forthcoming over the past decade, including now commercially available techniques such as desorption electrospray ionization (DESI),[1] direct analysis in real time (DART),[2] and atmospheric solids analysis probe (ASAP)[3] as well as the new inlet ionization methods of laserspray ionization (LSI)[4] and solvent assisted inlet ionization (SAII).[5] In spite of this flurry of activity in ionization methods, the now older techniques of electrospray ionization (ESI),[6] atmospheric pressure chemical ionization (APCI),[7] matrix assisted laser desorption/ionization (MALDI),[8] electron ionization, and chemical ionization[9] remain the dominant ionization methods used for organic compound analyses with MS. Among these methods, ESI and APCI are exceedingly popular, primarily because of their dominant position interfacing with liquid chromatography (LC)/MS.[10] The combined ability of ESI and APCI to analyze a wide range of materials containing small volatile low polarity to large nonvolatile highly polar compounds with good sensitivity provides a high bar for other ionization methods to exceed. Nevertheless, these methods have shortcomings such as selective ionization and ionization suppression which is especially onerous for mixtures, and improved sensitivity and ease of use is desirable for such goals as single cell analysis.

The initial introduction of LSI-MS by Trimpin et al. ushered in a series of new ionization approaches.[4,5,11−14] These ionization methods operating at intermediate pressure or high vacuum produce mass spectra, similar to ESI,[6] providing multiply charged ions from peptides and proteins as well as singly or multiply charged ions from a wide range of other compounds. One of these new ionization methods, SAII, has been shown to be more sensitive than ESI under similar flow rates for the compounds reported.[5,15−17] In SAII, as with the other inlet ionization methods, LSI[4] and matrix assisted inlet ionization (MAII),[12] ionization occurs in an inlet tube linking atmospheric pressure (AP) and the first vacuum stage of a mass spectrometer. SAII has been reported to be operable with LC/MS at solution flow rates as high as 55 μL min^{-1}[15] and as low as 400 nL min^{-1}.[16] Results were reported for a tryptic digest of bovine serum albumin with only 10 fmol injected on-column,[16] and for steroids similar ion abundances were achieved at 10× lower concentration in SAII relative to ESI.[17]

However, as noted in the original SAII paper, the method has some deficiencies.[5] The highest sensitivity is obtained with neutral pH solutions, but under these conditions, sodium adduction is common. Cation adduction can be minimized by using, for example, 0.1% formic acid but at the expense of sensitivity. This is less of a problem with LC/MS since the chromatographic resolution improves with acid addition gaining back much of the sensitivity loss. An advantage as well as a disadvantage of SAII is its selectivity, or lack of selectivity, relative to ESI. This can be problematic when

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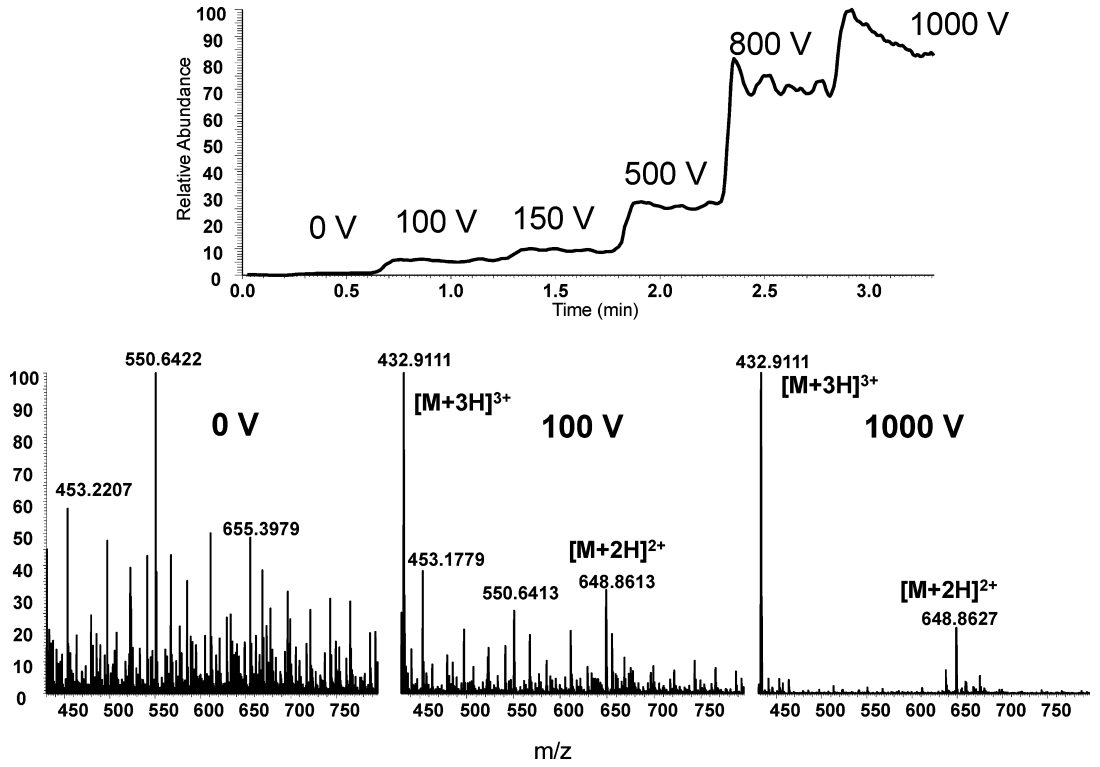

Figure 1. The effects of dc voltage on an analysis of a 96 nM solution of angiotensin II in 1:1 water/acetonitrile with 0.1% formic acid, mass spectra (bottom), and *m/z* 432.9 extracted ion chromatogram (EIC) (top).

analyzing low concentration samples, especially with liquid introduction by infusion, where polymer and other background compounds leached from containers during sample handling are more prominent in SAII than in ESI.

Here we describe a simple ionization approach, similar to SAII in which the solution is introduced either inside a heated inlet tube linking AP and the first vacuum stage of a mass spectrometer or just outside the inlet tube. Similar to ESI, a voltage is used to aid ionization of important classes of compounds. Combining this new approach with SAII provides selective ionization for different compound classes. For certain common samples analyzed by mass spectrometry, such as peptides and proteins, a factor of 10 to even 100-fold more ion abundance is observed with the voltage on relative to ESI using a commercial source. This increase in ion abundance is often at the expense of ionization of common contaminants, thus providing an exceptionally clean mass spectrum.

EXPERIMENTAL SECTION

A Thermo Fisher Scientific (Bremen, Germany) Orbitrap Exactive was used in these studies. The Ion Max ion source was removed and the interlock defeated to allow easy access to the inlet aperture of the inlet tube for SAII studies as previously reported.[1] A fused silica capillary (o.d. 220 μm, i.d. 150 μm, length 20 cm) had the exit-end, with the polyimide coating removed,[5] positioned inside the inlet tube using a manual *x,y,z*-stage so that its tip was near, or touching, the inlet tube inner wall and to a position inside the inlet that produced the highest abundance of the MH_2^{2+} ions of angiotensin II. The inlet temperature was 375 °C unless otherwise specified. The entrance end of the fused silica tubing was connected to a 30 cm length of either fused silica or PEEK (polyetheretherketone) tubing through a low dead volume union (Upchurch Scientific, 1/16, 10−32, 0.01 in.). The union could be either metal or PEEK polymer, but for polymer connectors only indirect application of voltage, as described below, is feasible. Solution was pushed through the tubing and union using a Chemyx (Thermo-Fisher Scientific) syringe pump flowing at 40 μL min^{-1}, although solution flow rates as low as 1 and as high as 100 μL min^{-1} also produced good results.

The metal union was attached to the instrument high voltage supply used for ESI operation. Voltage applied to the connector was between 100 V and 5 kV for positive ion operation and −100 V and −3 kV for negative ion operation. Typically, 1.5 kV was applied directly. Alternating current operating at 15 Hz, sine, was also applied with a maximum voltage of 575 V using an Agilent 33220A function generator and Trek model PZD700 high voltage amplifier. Alternatively, the voltage could be applied indirectly by application to a metal plate that was spaced from the metal connector by an air gap of about 2 mm. The nonconductive polymer union was placed inside brass tubing that was connected to a power supply as described below. Application of 300 V to 12 kV, employing a Hipotronics HV dc power supply (Brewster, NY), was used for these studies, but typically 2−3 kV produced optimum results.

In order to prevent operator contact with the high voltages used in these experiments, a nylon block was drilled so that a 1/2 in. long brass tubing with a high voltage cable attached fit snuggly inside and the union connector, metal or PEEK, was placed inside. The assembly eliminated the potential for direct contact with the high voltage. Additionally, the Hipotronics power supply was operated with the trip level set to the lowest current available.

Bovine insulin, angiotensin II, reserpine, acetonitrile, and Chromasolv HPLC water were purchased from Sigma Aldrich, St. Louis, MO. For all tests, the Orbitrap Exactive was tuned in

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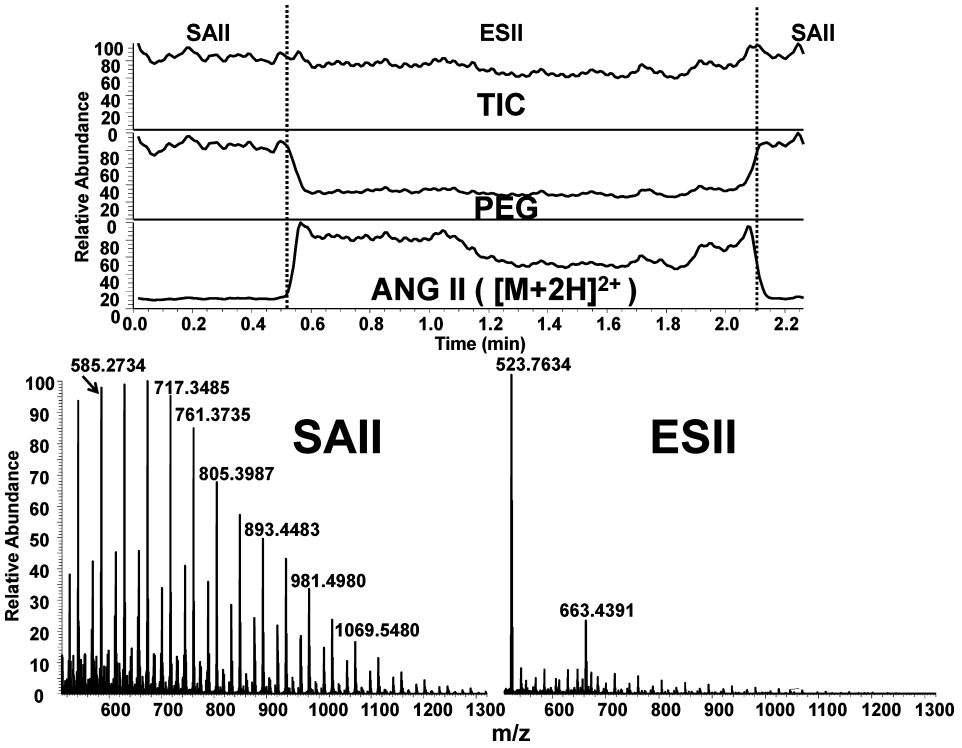

Figure 2. Comparison of SAII and ESII in a single analysis: TIC, m/z 585.2 (EIC), and m/z 523.8 EIC (top) and mass spectra (bottom).

the positive ion mode on the doubly protonated ion of Angiotensin II from a 100 nM 1:1 water/acetonitrile with 0.1% formic acid solution flowing at 40 μL min^{-1} using voltage applied to the PEEK union and with the inlet tube at 375 °C. For the negative ion spectra obtained with reserpine, an old instrument tune file obtained using ESI was employed.

RESULTS AND DISCUSSION

SAII provides exceptional sensitivity for a wide range of compound classes, possibly because the entire sample is introduced directly into the inlet of the mass spectrometer where ionization occurs at subambient pressure. With SAII, the inefficiencies of transferring ions from atmospheric pressure (AP) to vacuum are eliminated, but ion−ion recombination and neutralization at surfaces are expected to be more prominent than with AP ionization methods. While SAII appears to provide a more complete picture of the chemical components present in a solution relative to ESI (less ion suppression), it has disadvantages associated with impurities picked up in sample handling which provide a background that can mask the compound of interest when it is present in low concentration. Low molecular weight polymers, especially polyethylene oxides and polypropylene oxides, are observed in high abundance relative to ESI of the same solution. High-resolution mass spectrometers and inclusion of a prior separation device such as ion mobility spectrometry or LC reduces the contamination issue.

We now report that application of a voltage to the solution flowing into the heated inlet tube of the mass spectrometer significantly improves the ion abundance observed for certain important compound types such as peptides at the expense of ionization of many common contaminants. The method, referred to as electrosprayed inlet ionization (ESII) is applicable to either infusion introduction (see abstract graphic) or introduction of solution from a liquid chromatograph. How voltage is applied is important for good results, as is the position of the fused silica tubing exit end inside the instrument heated inlet tube. By connecting the tubing, either PEEK or fused silica, from a syringe or LC pump with the fused silica capillary used to introduce solution into the MS inlet tube using a metal or PEEK low dead volume union and applying a voltage either directly to the metal union, or indirectly to either the metal or polymer union, the ion abundance of peptides, proteins, and many small molecules are enhanced by up to 100-fold for some compounds relative to optimized SAII or ESI using the commercial probe on the Orbitrap Exactive Ion Max source.

Enhancement of signal is observed with application of as little as 100 V and increases with voltage, depending on the sample, to a maximum enhancement with application of between 1000 and 3000 V before decreasing or becoming unstable at higher voltage. Figure 1 shows the increase in ion abundance of a 100 fmol μL^{-1} solution of angiotensin II (Ang II) infused at 40 μL min^{-1} beginning at just 100 V relative to chemical background ions. The fused silica is positioned inside the inlet for optimum ESII results. At zero volts the ions for Ang II are embedded in the chemical background, but by 1000 V the ions from Ang II are well above any background ions.

A common contaminant in many samples, especially those stored in polymeric containers is low molecular weight polyethylene and polypropylene oxides. The relative sensitivity of SAII vs ESII for Ang II and polyethylene glycol (PEG) 1000 is shown in Figure 2. The fused silica capillary was adjusted inside the MS inlet tube to a position that worked well for both methods but was not optimum for either. Infusion of a 1:1:0.002 water/acetonitrile/formic acid solution containing 96 fmol μL^{-1} of Ang II and 135 ppb of PEG 1000 provided the same total ion current (TIC) with voltage on or off. However, plotting the ion abundances of the PEG mass-to-charge (m/z) 585.2 ion and that of the doubly charged ion of Ang II at m/z

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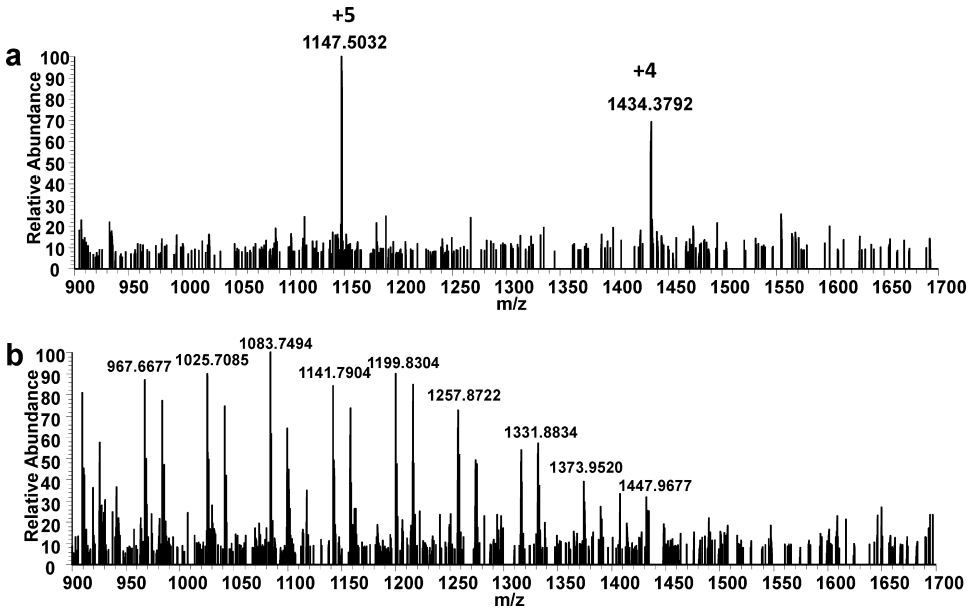

Figure 3. Single 0.1 s acquisition (180 fmol consumed) of a 1.72 nM solution of insulin in 1:1 water/acetonitrile with 0.1% formic acid at a flow rate of 40 μL min^{-1} and a capillary temperature of 375 °C (a) with voltage and (b) without voltage.

523.7 shows that SAII favors the PEG while ESII favors Ang II. Because voltage can be switched on and off, mass spectra from both methods can rapidly be obtained providing a more realistic picture of the compounds present in the solution.

It seems unlikely that the total ion current with ESII and SAII just happen to be the same suggesting that the instrumental maximum total ion current that can be transferred or detected is reached by both methods. This is supported by the finding that with ESII the ion abundance of peptides and proteins is only moderately affected by moving the exit end of the fused silica capillary outside of the inlet aperture by several millimeters. Providing a voltage to the solution outside the inlet tube opening would seem like standard ESI, but at the high flow rates and application of no desolvation gas, few of the charged droplets are desolvated before the inlet entrance. We speculate that within the inlet tube, the heat and pressure drop provide a mechanism for enhancing formation of small, highly charged droplets by the same process that produces charged droplets in SAII. This process of droplet formation by rapid boiling may produce very small charged droplets similar to nanoESI and account for some of the increased sensitivity relative to ESI.

The sensitivity of ESII can be seen for insulin in Figure 3 for a single 0.1 s acquisition of a 1.7 nM solution flowing at 40 μL min^{-1} (180 amol consumed). Figure 3b obtained at the optimum applied voltage position (not the optimum SAII position) is without the voltage and shows primarily $[M + Na]^+$ peaks of polyethylene oxide. Figure 3a is the spectrum observed with 2000 V applied directly to a metal connector showing dominantly multiply charged protonated molecular ions of insulin. ESII is also more sensitive than optimized ESI operated using the heated ESI (HESI) probe of the Ion Max source. A direct comparison using the same Ang II solutions showed up to 100× more total Ang II ion abundance under similar acquisition conditions for ESII relative to ESI over a wide concentration range. While proteins also provide more ion abundance using ESII relative to ESI or SAII, the ion abundance differences are much smaller than for peptides. Some small molecules, usually more basic compounds, also show enhancement with ESII relative to ESI or SAII, but a number of compounds are either not enhanced or show lower sensitivity than SAII.

Just as with SAII, without addition of acid, substantial sodium adduction is observed with ESII, but, with 0.1% formic acid, the sodium adducts are almost eliminated and the protonated multiply charged peaks dominate. The ESII method has other characteristics of SAII. The best results for flow rates above a few μL min^{-1} are obtained when the exit end of the fused silica capillary is inside the inlet tube and adjusted to touch or be very close to the inlet wall. At flow rates below a few μL min^{-1}, similar results are obtained with the tip of the fused silica outside the inlet tube, as in microflow ESI, or slightly inside the inlet tube. Ionization also improves with inlet temperature in the same manner as with SAII.[15] A sizzling or rapid popping sound heard for SAII operation is also noted with ESII but with a higher frequency. Finally, the heated inlet allows the method to be operational over the same flow rates observed in SAII reaching a broad maximum on the Orbitrap Exactive at about 60 μL min^{-1}.

The means of voltage application is important, but not understood at this time. For example, placing a voltage directly on a solution being drawn through the SAII fused silica capillary provides an increased signal that drops off in abundance sometimes to the point that no signal is observed. The same is true if the voltage is placed directly on a syringe needle through which the solution is pushed into the SAII fused silica capillary. However, if a voltage is placed on a metal plate above a droplet but not in direct contact with the droplet placed on Teflon, the ion abundance increases and remains steady. A similar enhancement and steady ion current is obtained for flowing solutions from an LC or by infusion by applying the voltage directly or indirectly to a metal or PEEK union as described above.

Unlike with ESI, there is no onset voltage when the ion enhancement suddenly occurs. Some effect can be observed at <100 V and continues to increase with increased voltage to between 1.0 and 3.0 kV. We attribute this to the voltage providing a mechanism of charge separation in the solution and

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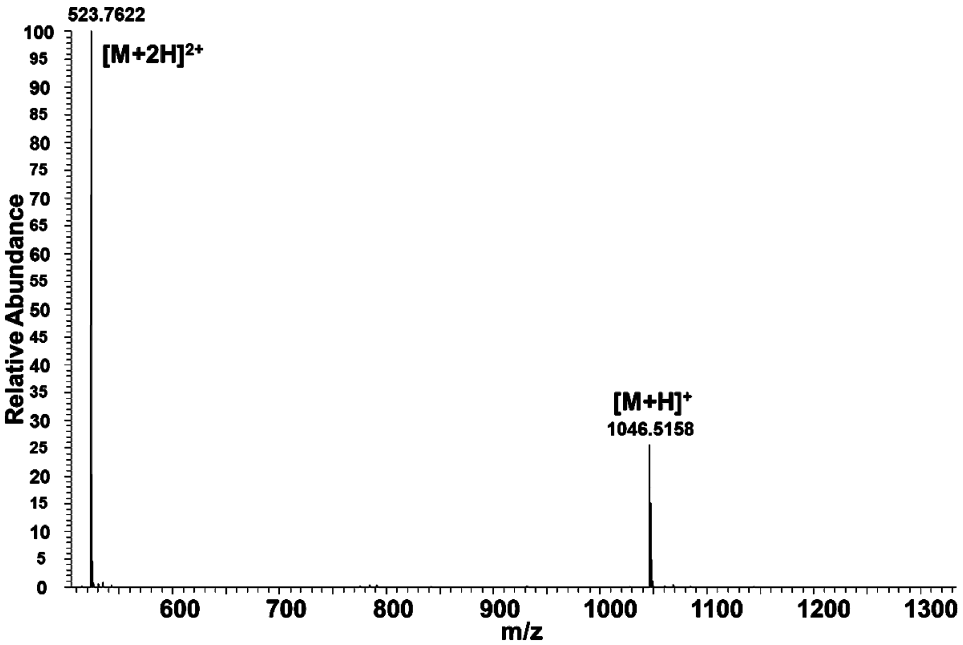

Figure 4. ESII spectrum of angiotensin II obtained with a voltage of 575 V ac (15 Hz sine waveform).

rapid solution boiling inside the hot inlet tube providing a mechanism for droplet formation, whereas in ESI, the onset voltage is related to having a sufficient field to induce Taylor cone formation in order to produce the highly charged droplets. Further, as has been reported for ESI, an ac voltage can be used to provide signal enhancement.[18] Using 15 Hz (sine) and the maximum voltage available from the power supply, 575 V ac, the mass spectrum shown in Figure 4 was obtained for Ang II. The signal enhancement relative to no voltage (not optimized for SAII) was almost 1000×. ESII also operates in the negative mode, similar to ESI.

ESI has been the gold standard for ionization for mass spectrometry in a wide range of fields, but most notably the method was an important driver for innovations in biological research. The ability to obtain different selectivity with the voltage on (ESII) or off (SAII) is expected to have analytical utility, and on the basis of our early results, we expect ESII/SAII to find wide applications because of the high sensitivity and ease of use relative to ESI.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Takats, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. *Science* **2004**, *306*, 471−473.
(2) Cody, R. B.; Laramee, J. A.; Durst, H. D. *Anal. Chem.* **2005**, *77*, 2297−2302.
(3) McEwen, C. N.; McKay, R. G.; Larsen, B. S. *Anal. Chem.* **2005**, *77*, 7826−7831.
(4) Trimpin, S.; Inutan, E. D.; Herath, T. N.; McEwen, C. N. *Mol. Cell. Proteomics* **2010**, *9*, 362−367.
(5) Pagnotti, V. S.; Chubatyi, N. D.; McEwen, C. N. *Anal. Chem.* **2011**, *83*, 3981−3985.
(6) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64−71.
(7) Carroll, D. I.; Dzidic, I.; Stillwell, R. N.; Haegele, K. D.; Horning, E. C. *Anal. Chem.* **1975**, *47*, 2369−2373.
(8) Karas, M.; Bachman, D.; Bahr, U.; Hillenkamp, F. *Int. J. Mass Spectrom.* **1987**, *78*, 53−68.
(9) Munson, M. S. B.; Field, F. H. *J. Am. Chem. Soc.* **1966**, *88*, 2621−2630.
(10) Kostiainen, R.; Kotiaho, T.; Kuuranne, T.; Auriola, S. *J. Mass Spectrom.* **2003**, *38*, 357−372.
(11) Trimpin, S.; Inutan, E. D.; Herath, T. N.; McEwen, C. N. *Anal. Chem.* **2010**, *82*, 11−15.
(12) McEwen, C. N.; Pagnotti, V. S.; Inutan, E. D.; Trimpin, S. *Anal. Chem.* **2010**, *82*, 9164−9168.
(13) Inutan, E. D.; Wang, B.; Trimpin, S. *Anal. Chem.* **2011**, *83*, 678−684.
(14) Trimpin, S.; Ren, Y.; Wang, B.; Lietz, C. B.; Richards, A. L.; Marshall, A. G.; Inutan, E. D. *Anal. Chem.* **2011**, *83*, 5469−5475.
(15) Pagnotti, V. S.; Inutan, E. D.; Marshall, D. D.; McEwen, C. N.; Trimpin, S. *Anal. Chem.* **2011**, *82*, 7591−7594.
(16) Wang, B.; Inutan, E. D.; Trimpin, S. *J. Am. Soc. Mass Spectrom.* **2012**, *23*, 442−445.
(17) Chubatyi, N. D.; Pagnotti, V. S.; Bentzley, C. M.; McEwen, C. N. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 887−892.
(18) Chetwni, N.; Cassou, C. A.; Go, D. B.; Chang, H.-C. *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 1852−1856.