Published in final edited form as:

J Am Soc Mass Spectrom. 2014 April ; 25(4): 524–529. doi:10.1007/s13361-013-0809-4.

A Comparison of Alternating Current and Direct Current Electrospray Ionization for Mass Spectrometry

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Abstract

A series of studies comparing the performance of alternating current electrospray ionization (AC ESI) mass spectrometry (MS) and direct current electrospray ionization (DC ESI) MS has been conducted, exploring the absolute signal intensity and signal-to-background ratios produced by both methods using caffeine and a model peptide as targets. Because the high-voltage AC signal was more susceptible to generating gas discharges, the operating voltage range of AC ESI was significantly smaller than that for DC ESI, such that the absolute signal intensities produced by DC ESI at peak voltages were 1 - 2 orders of magnitude greater than those for AC ESI. Using an electronegative nebulizing gas, sulfur hexafluoride (SF₆), instead of nitrogen (N₂) increased the operating range of AC ESI by ~50%, but did not appreciably improve signal intensities. While DC ESI generated far greater signal intensities, both ionization methods produced comparable signal-to-background noise, with AC ESI spectra appearing qualitatively cleaner. A quantitative calibration analysis was performed for two analytes, caffeine and the peptide MRFA. AC ESI utilizing SF₆ outperforms all other techniques for the detection of MRFA, producing chromatographic limits of detection nearly one order of magnitude lower than that of DC ESI utilizing N₂, and one half that of DC ESI utilizing SF₆. However, DC ESI outperforms AC ESI for the analysis of caffeine, indicating improvements in spectral quality may benefit certain compounds, or classes of compounds, on an individual basis.

Introduction

Since its development, conventional direct current electrospray ionization (DC ESI) has been coupled to mass spectrometry as a mainstay technique for the analysis of a wide variety of biomolecules. Its relative softness compared to fast atom bombardment (FAB) and

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electron impact (EI) ionization, as well as its ability to ionize very large biomolecules, have made it the ionization method of choice for modern proteomic studies alongside matrix-assisted laser desorption ionization (MALDI)[1,2]. A new method of electrospray ionization based on alternating current (AC ESI) has recently been described [3,4], and initial results showed that it produced up to an order of magnitude increase in signal intensity over conventional DC ESI for intact protein analytes under certain spray conditions. However, a detailed study and analysis of the operational voltage range of AC ESI as compared to DC ESI has not yet been conducted, nor a realistic assessment of the operating conditions when AC ESI outperforms or does not outperform DC ESI. In this work, we explore a wide voltage operating space and the role of the nebulizing gas to determine the relative merits of AC ESI.

In AC ESI, a sinusoidal potential at a frequency of approximately 80 - 400 kHz is applied directly to a stainless steel electrospray emitter through which the analyte solution is pumped, while the mass spectrometer inlet is grounded. Cone formation for AC ESI is significantly different from that for DC ESI, as confirmed by both experiment and theory [5,6], due to a mechanism termed “preferential entrainment.” This process can best be understood by considering the two half cycles separately during the AC ESI of an acidic protein solution. During the half cycle in which the spray emitter is at positive potential, more electrophoretically-mobile protons and less-mobile acidified proteins are attracted toward the tip of the spray cone. When the polarity is reversed, the more mobile protons can be driven back into the bulk solution by the electric field. If the cycle duration is relatively short (*i.e.* at high frequencies), there is insufficient time for the more massive proteins to relax away from the high electric field at the spray tip before the polarity is again reversed. Both sets of species are forced toward the cone tip by the bulk solution flow. In this way, bulky analyte molecules are entrained near the spray cone meniscus where the electric field is high and the proteins become ionized. After many cycles, repulsion forces between accumulated protein ions expel them in a narrow spray axial to the cone. The result is that the AC ESI cone is much narrower than the DC ESI cone with a half angle of approximately 12° for AC ESI [5,6] compared to 49° for DC ESI [7,8]. The preferential entrainment of analyte ions near the cone meniscus and narrower spray cone shape are thought to improve sensitivity during AC versus DC ESI. Though the spray droplets emitted from an AC ESI cone are larger and carry less charge than those from DC ESI, the combination of preferential entrainment and the axial emission of droplets is presumed to be the main cause of increased sensitivity [3].

Previous work had focused on comparing optimized spray conditions for AC ESI to equivalent conditions in DC ESI [3]. Among a number of other parameters, this entailed application of a DC potential that was equal to the root-mean-square (RMS) of the applied alternating potential ($V_{DC} = V_{AC,RMS}$). It was under these equivalent conditions that the reported order-of-magnitude absolute signal intensity improvement was observed. However, the potential range accessible during AC ESI is limited because gas discharges occur at lower potentials under AC conditions than DC [9], and previous studies reported a range from approximately 300 -1500 V_{RMS} in which signal decreased as the frequency increased [4]. Therefore, prior comparisons employed a DC ESI potential of $V_{DC} \sim 1 - 1.5$ kV, which

is significantly lower than typical DC ESI potentials (2 - 3 kV) for emitters at a fixed distance from the mass spectrometer inlet. Thus, while AC ESI showed greater absolute signal intensity than DC ESI at comparable conditions, the limitations of operating AC ESI at higher voltages prevented a comparison of its overall value compared to DC ESI. Recently, contactless methods of applying electrospray potential, including square-wave and AC potentials, have been described. [10-11] These contactless methods reduce gas discharge limitations and allow the application of potentials in excess of 6 kV, and as such, produce improved sensitivity over DC ESI. In this study, however, an insulating nebulizing gas is used to extend voltage range instead, as this change is easily incorporated into already existing emitter designs.

In this work, we report a comparison of AC ESI and DC ESI under applied voltage and nebulizing gas conditions individually optimized for each technique. In general, AC ESI only produced higher signal intensities at relatively low applied voltages, and they were significantly lower than those observed under DC ESI conditions when both were independently optimized. However, it was found that they produce comparable signal-to-background noise ratios under most conditions examined.

Experimental

Mass spectra were collected using a linear ion trap mass spectrometer (LTQ-XL, Thermo Fisher Scientific, Waltham, MA) operating in normal mass range mode. Electrospray was conducted by direct infusion at a rate of 500 nL/min via the instrument's syringe pump through a stainless steel emitter with a 50 μ m internal diameter (New Objective, Woburn, MA) mounted axial to the inlet at a distance of 5 mm and electrically isolated from the instrument. Nitrogen (N₂) or sulfur hexafluoride (SF₆) gas was supplied at varying pressures as specified in the results to act as a nebulizing gas in both AC and DC sprays. The mount, emitter, and nebulizing gas setup were identical for both AC and DC spray conditions.

To generate AC sprays, AC potential was applied directly to the steel emitter using a function generator (model 33220A, Agilent, Santa Clara, CA) connected to a radio frequency (RF) amplifier (500A, Industrial Test Equipment Co., Port Washington, NY) and a custom-made transformer (Industrial Test Equipment Co., Port Washington, NY). The applied potential was monitored using a high-voltage probe (P6105, Tektronix Inc., Beaverton, OR) and oscilloscope (PM3375, Koninklijke Philips Electronics N.V., Amsterdam, Netherlands). To generate DC sprays, DC potential was applied directly to the emitter using an external power supply (model ES-5R1.2, Matsusada Precision, Bohemia, NY). In both cases, the mass spectrometer end plate was kept at ground potential (0 V).

In our comparative studies of AC and DC ESI, we used instrument tune mix recommended by Thermo-Fisher consisting of caffeine (20 μ g/mL, 100 μ M), the tetrapeptide methionine-arginine-phenylalanine-alanine (MRFA, 15 μ M, 7.9 μ g/mL), and a mixture of fluorinated phosphazines, Ultramark 1621 (0.01%), in an aqueous solution of acetonitrile (50%), methanol (25%), and acetic acid (1%). This solution was used to tune ion optics parameters and to calibrate mass and resolution parameters prior to optimizing spray conditions. To carry out limit-of-detection (LOD) analyses, serial dilution series of caffeine (12.5 – 100

$\mu\text{g/mL}$) and MRFA ($1.25 - 10 \mu\text{g/mL}$) were prepared. To each dilution, a fixed amount of the amino acid asparagine ($62.7 \mu\text{M}$) was added to act as an internal standard. A blank sample was created to which only the internal standard asparagine was added. The dilutions were analyzed in random order and in triplicate.

Ion optic tuning parameters were constant across both ionization methods, and forty scans of the infused calibration mix were collected and averaged for each experiment. The automatic gain control target was set to 30,000 with a maximum inject time of 10 ms. Three microscans were averaged per spectrum. All studies were conducted in positive ion mode.

Results and Discussion

While prior studies compared AC and DC ESI performances at equivalent conditions that were only optimized for AC ESI [3], in this work the first aim was to individually optimize each spray technique, thus ensuring a more realistic comparison of likely performance. To do so, we compared both the operating range and the nebulizing gas conditions for both spray methods.

Using N_2 at a pressure of 4.1 bar as the nebulizing gas, the frequency of AC ESI was first optimized by varying it over a range of $f = 80 - 200 \text{ kHz}$ and assessing the applied voltage range over which the peak corresponding to the protonated peptide MRFA ($m/z = 524.2$) was observable before a gas discharge occurred (see Supplemental Material). Consistent with prior studies [4], the optimal frequency was found to be $f = 120 \text{ kHz}$. Stable mass spectra were produced over the range of $V_{AC} = 200 - 1000 \text{ V}$, where V_{AC} is defined as the amplitude of the applied AC potential. Figure 1 shows a comparison of intensity across the AC voltage range to DC ESI with all other parameters (flow rate, N_2 nebulizing gas pressure, and mass spectrometer settings) identical. In this comparison, we specify that the applied potential is equivalent when the DC potential (V_{DC}) is the same as the *amplitude* of the AC potential (V_{AC}) whereas earlier studies specified equivalence as matching the RMS of the AC potential. We revise this comparison here because AC ESI can be effectively analyzed based on a quasi-static half cycle [5], suggesting that the ionization is strongly tied to the peak electric field that corresponds to the voltage amplitude in the half-cycle. The absolute signal intensity of MRFA ($m/z = 524.2$) for both AC and DC ESI increased with increasing voltage, and AC ESI produced a similar spectral intensity to DC ESI at equivalent applied potentials in the range of $V_{AC} = 200 - 1150 \text{ V}$. Beyond this voltage, the signal intensity for the base peak decreased as the AC voltage was continuously increased until reaching discharge at 1350 V. However, DC ESI did not reach its discharge limit until $V_{DC} = 2700 \text{ kV}$ was applied to the emitter. Because of the lower discharge limit of AC ESI, DC ESI produced an absolute signal intensity of approximately $50\times$ the intensity of AC ESI at voltages not accessible by AC ESI. For example, the maximum signal intensity at the maximum voltage of $V_{AC} = 1150 \text{ V}$ was only 2% of that achieved by DC ESI at its maximum operating voltage, $V_{DC} = 2700 \text{ V}$. While only results for MRFA ($m/z = 524$) are discussed here, similar trends are observed for all analytes in the tune mix and further detail is presented in Supplementary Materials.

A brief study of the application of a DC bias on the applied AC potential was performed utilizing N₂ as a nebulizing gas. DC bias was applied by setting the internal power supply of the mass spectrometer to a positive or negative potential to apply a bias. When a relatively small DC bias was applied, the AC discharge limit was reached at a lower potential equivalent to the applied DC bias, and spectral quality degraded. When a larger DC bias was applied, very little AC potential could be applied on top without inducing discharge, and the spectra greatly resembled DC ESI spectra with no superimposed AC signal. Stable spray without discharge was difficult to maintain across all tested voltage conditions.

It is well known that electronegative gases can delay the initiation of a gas discharge [9], and for this reason they have been used in negative-ion mode DC ESI to extend analysis capability [12]. In order to extend the operational voltage range of AC ESI, we used SF₆ as the nebulizing gas. In this way, we were able to extend the applied potential before signal reduction to 1850 V at 120 kHz, an increase in accessible voltage greater than 50% that achievable using N₂ as nebulizing gas. However, as shown in Figure 2a, while the operating range was increased, the overall signal intensities for AC ESI were still significantly lower than for DC ESI. For example, at $V_{AC} = 1150$ V in N₂, the AC ESI peak intensity for the peptide MRFA was 2.2×10^4 , whereas at $V_{AC} = 1850$ V in SF₆, the AC ESI peak intensity was 3.3×10^4 . Further, both of these were lower than the DC ESI peak intensities at their maximum voltage, which were 1.0×10^6 at 2600 V in N₂, and 2.1×10^5 at 3200 V in SF₆.

Figure 2b shows the calculated signal-to-background (*S/B*) ratios for the peak corresponding to MRFA as a function of the operating voltage for AC and DC ESI using both N₂ and SF₆ as nebulizing gasses. Here, we refer to, “signal-to-background” to distinguish the fact that the noise is reduced across the *m/z* dimension, as opposed to, “signal-to-noise,” which typically describes peak quality in a chromatograph. For the operating range of both methods, the *S/B* is approximately the same, even though the DC ESI absolute signal intensity is much stronger for the same conditions as shown in Figure 1. Importantly, the *S/B* for AC ESI surpassed that of DC ESI, being 54% greater at its peak value using SF₆ as nebulizing gas ($V_{AC} = 1250$ V) than that of DC ESI at its peak value in SF₆ ($V_{DC} = 3100$ V). Another interesting feature of the *S/B* behavior comparison was a difference in how the maximal values were achieved under the two ionization methods. As the applied potential in DC ESI approached its discharge limit, the *S/B* appeared to have settled at a maximum value in spite of the fact that the absolute signal intensity was still rapidly increasing. This suggests that applying potentials greater than approximately 1200 V in DC ESI produces more signal, but also produces more background chemical noise in an equal amount to signal with respect to applied potential. However, the *S/B* did not appear to approach a plateau under AC ESI conditions as voltage was increased. Rather, the *S/B* increased with voltage, then sharply decreased, which may be evidence for the discharge limit. However, this evidence for the discharge regime appears at even lower voltage in the *S/B* plot than in the absolute intensity plot in Figure 1 and Figure 2. For example, in the absolute intensity plot, the discharge limit for AC ESI in SF₆ appears to initiate at $V_{AC} = 1900$ V. In the *S/B* plot, the ratio starts to rapidly decrease at about $V_{AC} = 1300$ V. This indicates that AC ESI continues to produce more detectable ions up to 1900 V, but weak discharge or other effects begin to create interfering background ions at earlier voltages.

Despite AC ESI operating over a smaller voltage range and producing less absolute signal intensity, the general quality of AC ESI mass spectra appeared to be superior to those acquired under DC ESI conditions. Figure 3a shows intensity-normalized spectra for both AC and DC ESI under optimized voltages utilizing SF₆ as nebulizer. The relative intensity of the background signal was much smaller using AC ESI, indicating that it produces less noise than its DC counterpart by suppressing background peaks. In order to characterize this effect statistically, cumulative distribution functions for the relative signal intensities under both AC and DC ESI conditions were calculated (Figure 3b). For AC ESI, 90% of the relative intensity values in the spectrum were less than 0.14% of the base peak intensity. DC ESI resulted in 90% of the spectral intensity values being less than 0.18% of the base peak intensity. These observations suggest that, with respect to *S/B* ratios, AC ESI produces spectra similar or superior to those acquired using DC ESI, in spite of the fact that AC ESI produces much lower overall signal intensities. To quantify *S/B*, signal was statistically separated from background by utilizing an outlier rejection algorithm. In this way, *m/z* intensity values that fell greater than four standard deviations away from the background mean were rejected. Since intensity values corresponding to background dominate mass spectrometry data, the rejected intensity values are signal and not related to background ion effects, experimental variability, or other uncontrollable signal sources. The standard deviation of the remaining intensity values represents an objective measurement of background in the mass spectrum (see Supplementary Material for details on this calculation).

The similar *S/B* ratio for AC ESI and DC ESI suggests that, even if the absolute signal intensity of AC ESI is less than that of DC ESI, they should have similar quantitative behavior in terms of the concentration range over which a target signal can be distinguished from background noise. To evaluate this hypothesis, calibration curves were generated for both caffeine and MRFA in AC ESI using both N₂ and SF₆ as nebulizing gasses, where chromatographic signal-to-noise ratio is plotted as a function of analyte concentration. From these calibration curves, limits of detection were estimated for MRFA and caffeine. As conjectured, AC ESI and DC ESI perform very similarly for MRFA when utilizing N₂ (Table 1). Switching to SF₆ significantly improves detection limits for MRFA in both techniques. However, this effect is more prominent in AC ESI resulting in a detection limit half that of DC ESI utilizing SF₆, and nearly an order of magnitude less than that for DC ESI utilizing N₂. For caffeine, however, DC resulted in better limits of detection than AC ESI for both nebulizing gasses. Switching to SF₆ did not produce significant improvement in limits of detection for caffeine in either AC ESI or DC ESI. This suggests that the performance of each method depends, to some extent, on the physical properties of the analytes and their individual potentials to ionize.

Conclusion

In this work, a comparison of AC to DC ESI showed that because of the larger operating voltage range, DC ESI produces spectra with significantly more intense signal than does AC ESI. However, at these voltages, the relative intensity of the background noise is also higher, such that both AC ESI and DC ESI produce spectra with similar signal-to-background ratios. For this reason, although AC ESI does not produce signal intensity with magnitudes

as great as those observed during DC ESI, they have similar quantitative capability. For one tested analyte utilizing SF₆ as a nebulizing gas, AC ESI outperforms DC ESI, producing spectra with a limit of detection for the peptide MRFA that is one half that of DC ESI utilizing SF₆. However, DC ESI outperforms AC ESI for the analysis of caffeine. Therefore, the physical characteristics of the analyte dictate the best ionization method for qualitative and quantitative applications. Further study is needed to determine whether or not there are general classes of compounds for which one technique outperforms the other.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Drs. William Boggess and Michelle Joyce of the University of Notre Dame Mass Spectrometry and Proteomics Facility for their assistance on this project. NJD acknowledges support from a grant from the National Institutes of Health (R01GM096767).

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Figure 1.

(a) Absolute signal intensity of MRFA ($m/z=524$) as a function of the applied voltage for AC ESI and DC ESI. (b) Signal to background ratio as a function of the applied voltage for AC ESI and DC ESI. For all these experiments, N_2 was used as the nebulizing gas at 4.1 bar and the AC ESI frequency was 120 kHz

Figure 2.

(a) Absolute signal intensity of MRFA ($m/z=524$) as a function of the applied voltage for AC ESI and DC ESI using both N_2 and SF_6 nebulizing gasses at 4.1 bar. The inset magnifies the region below 2000 V. (b) Signal-to-background ratio of MRFA as a function of the applied voltage for AC ESI and DC ESI. For all AC ESI experiments the frequency was 120 kHz

Figure 3.

(a) Mass spectra of caffeine ($m/z=195$) and MRFA ($m/z=524$) in DC ESI (top) and AC ESI (bottom) at each techniques' respective optimum potential illustrating the reduced intensity of background evident in AC ESI. (b) Cumulative distribution function of AC ESI and DC ESI intensity data under optimum conditions for both techniques utilizing SF_6 as nebulizing gas. Spectral data were normalized to the intensity of caffeine before analysis

Table 1

Limits of detection calculated for caffeine ($m/z=192$) and MRFA ($m/z=524$) in AC ESI and DC ESI at a variety of voltages and utilizing both N₂ and SF₆ as a nebulizing gas

Technique	Nebulizing Gas	Voltage (kV)	Caffeine LOD ($\mu\text{g/mL}$)	MRFA LOD ($\mu\text{g/mL}$)
DC ESI	N ₂	2.0	0.33 $\pm$ 0.04	0.5 $\pm$ 0.1
	N ₂	2.4	0.16 $\pm$ 0.01	0.36 $\pm$ 0.02
	SF ₆	2.4	0.24 $\pm$ 0.03	0.09 $\pm$ 0.02
	SF ₆	3.0	0.166 $\pm$ 0.007	0.11 $\pm$ 0.01
AC ESI	N ₂	1.2	0.47 $\pm$ 0.08	0.30 $\pm$ 0.03
	SF ₆	1.2	0.52 $\pm$ 0.09	0.052 $\pm$ 0.002
	SF ₆	1.6	0.43 $\pm$ 0.06	0.073 $\pm$ 0.002