

Multiply-charged Ion Formation by Sonic Spray

Atsumu Hirabayashi,* Yukiko Hirabayashi, Minoru Sakairi and Hideaki Koizumi

Central Research Laboratory, Hitachi, Ltd, Kokubunji, Tokyo 185, Japan

In sonic spray ionization technique, a solution from a fused-silica capillary is sprayed with a sonic gas flow coaxial to the capillary. The current for the ions produced and for the charged droplets is found to be dramatically enhanced when a voltage is applied to a surrounding metal piece, which is isolated from the solution by the fused-silica capillary. This can be ascribed to the induced concentration difference between positive and negative ions in the solution near the surface. Furthermore, multiply-protonated molecules produced in this way from protein solutions are analyzed with a quadrupole mass spectrometer. The ion intensity shows a strong dependence on the voltage but the diameter of the droplet produced by the spray is likely to be independent of the voltage. Thus, we conclude that the charge density of the droplet is regulated by the voltage.

Recently, sonic spray ionization has been developed as a novel spray ionization technique for mass spectrometry.¹ In this technique, a solution of methanol/water is sprayed from a fused-silica capillary with gas flow coaxial to the capillary. Although the sprayed gas is electrically neutral, ions and charged droplets are produced at atmospheric pressure. The intensity of the ion current depends on the gas velocity and is a maximum near to sonic gas velocity. In this technique, singly and doubly charged ions of biological molecules are readily produced with high efficiency. In particular, we have obtained mass spectra from a dopamine solution at a 10 nM concentration.²

The origin of the charged species is ascribed to non-uniformity of ion concentrations at the solution surface: on the surface of pure water, for example, a negatively charged layer is formed with a positively charged layer underneath. The depth of the double layer is of the order of 10 nm, although this depends on the ion concentration.³ Therefore, the distributions of positive and negative ions are quite different at the liquid surface. When such a surface is disrupted by a shear stress, charge separation occurs and charged droplets are produced.

Since the non-uniformity of ion concentration at the solution surface is determined by the surface potential, it may be regulated by applying an electric field to the solution. When the charge density of the droplet initially produced is increased, the ion formation efficiency from the charge droplet should be increased. In this study, we report the generation of a charged, sprayed gas flow by applying an electric field to the solution and present preliminary results for cytochrome c and myoglobin.

EXPERIMENTAL

A cross-sectional view of the sonic spray ion source is shown in Fig. 1. Methanol/water (50/50%, v/v) containing a variable amount of acetic acid was pumped by a syringe

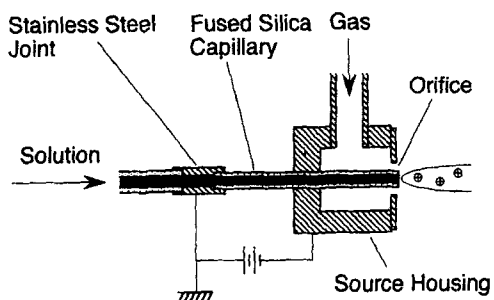


Figure 1. Cross-sectional view (schematic) of the ion source.

* Author for correspondence.

pump (Model 11, Harvard Apparatus, South Natick, MA, USA) at a flow rate of 30 $\mu\text{L}/\text{min}$ through a stainless steel joint, which was held at ground potential, to a fused-silica capillary (0.1 mm i.d., 0.2 mm o.d.). The capillary tip was inserted through a Duralumin orifice (0.4 mm i.d.) in the Duralumin housing, with the tip extending 0.2 mm beyond the orifice. A voltage was applied to the housing which was electrically isolated from the solution by the fused-silica capillary. The housing was pressurized with nitrogen which caused a gas flow through the orifice at a rate that could be measured with a mass flow controller (5850E, Brooks Instrument, Halfield, PA, USA). A spray was thus generated in which ions and charged droplets were produced. A quadrupole mass spectrometer was operated at 2.6×10^{-4} Pa to analyze the ions produced by the spray; the details of the mass spectrometer has been described elsewhere.¹ The distance between the fused-silica capillary tip and the sampling orifice of the mass spectrometer was 5 mm, and their central axes were aligned.

Deionized water was used to prepare solutions. Methanol and acetic acid were purchased from Wako (Tokyo, Japan). Trifluoroacetic acid and sodium hydroxide were from Nakarai (Kyoto, Japan). Cytochrome c and myoglobin were from Sigma Chemical (St. Louis, MO, USA). All chemicals were used without further purification.

RESULTS AND DISCUSSION

Ion current regulated by applied voltage

A 155-mm long stainless steel tube with an inner diameter of 35 mm was used as an electrode to detect all the charged particles produced by the spray, where the exit end of the tube was covered with a Cu mesh. The current between the stainless steel tube and ground was measured with an ammeter.

In Fig. 2 the ion current detected at the stainless steel tube is shown as a function of the voltage V applied to the source housing. The results were obtained from methanol/water which contained 1%, 5% and 20% acetic acid. From the figure we conclude the following: (1) the ion current at $V=0$ is zero, (2) for $|V| < 1.2$ kV the ion current decreases with increasing voltage, (3) for $|V| > 1.2$ kV the current is almost independent of the voltage, and (4) the ion current increases with increasing acetic acid concentration. Since the pH of the 5% acetic acid solution was measured to be 2.5, which corresponds to the proton concentration for a 3 mM solution of a strong electrolyte, we have tried 3 mM solutions of NaOH and trifluoroacetic acid. The results are similar to those shown in Fig. 2. Therefore, we conclude that the non-

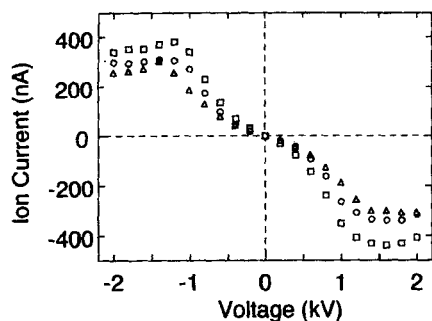


Figure 2. Ion current as a function of the voltage applied to the source housing. The results were obtained from 1% (Δ), 5% (\circ) and 20% (\square) acetic acid solutions of methanol/water and with a gas-flow rate of 3.0 L/min.

uniformity in ion concentration at the solution surface is regulated by an applied electric field. At voltages above 1.2 kV, the concentration of ions of the same polarity is likely to be saturated at the solution surface. Note that positively and negatively charged particles are produced at $V=0$; although the droplet initially produced by the spray might not be charged, the positive and negative ion concentrations in the droplet are different near the surface because of the surface potential, and small charged droplets are produced from the droplet by the gas flow. (This charged droplet formation cannot be ascribed to the statistical charging model,^{1,4} which is accepted for the mechanism of charged droplet formation in thermospray ionization.⁵) On the other hand, when a specific voltage is applied, the initial droplet will be charged.

Figure 3 shows the gas-flow rate dependence of the ion current when a voltage of -1 kV is applied to the source housing. At a gas-flow rate below 0.3 L/min, the spray is unstable. From the figure, we conclude the following: (1) the ion current increases with increasing gas-flow rate below 3.0 L/min and (2) the ion current is almost unchanged at gas-flow rates higher than 3 L/min. The gas velocity and therefore the Mach number increase with increasing gas-flow rate, although their relation is not clear in our experiment, as described in our previous publication.² It has been confirmed by the Schlieren method that at a gas-flow rate of 3.0 L/min the Mach number is about 1 (sonic velocity). Furthermore, at 6.1 L/min the Mach number is estimated to be less than 2.

When a droplet is undergoing separation from the solution surface, an ion flow occurs from the solution surface to neutralize the droplet. The relaxation time for this neutralization, which is independent of the droplet size,⁶ is roughly estimated to be 10^{-7} s. This is much longer than the

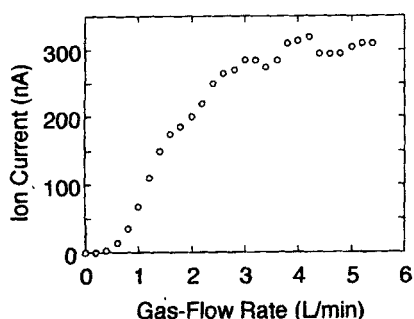


Figure 3. Ion current as a function of the gas-flow rate. These data were obtained when a high voltage of -1 kV was applied to the source housing. With increasing gas-flow rate, the gas velocity increases and at 3.0 L/min it is about sonic.²

disruption time of 3×10^{-9} s, assuming a droplet diameter of $1 \mu\text{m}$ and a relative velocity of the nitrogen gas which is the sonic velocity (3×10^2 m/s). Therefore, this effect is negligible.

On the other hand, the charge density in a droplet is expected to increase with decreasing droplet diameter because of the non-uniformity in the ion concentrations. Since the gas velocity is related to the pressure gradient around the fused-silica capillary tip, the droplet diameter is likely to depend on the pressure gradient. In Fig. 3, the increase in ion current with increasing gas velocity in the subsonic region can be ascribed to the decrease in droplet diameter. However, in the supersonic region, the ion current does not increase. This can be ascribed to the shock wave generated in the supersonic flow. When the shock wave is generated, the pressure gradient around the capillary tip becomes unstable. Thus, the formation of fine droplets is rather suppressed.

Multiply-charged ion formation

Figure 4 shows typical examples of the mass spectra obtained from the $1 \mu\text{m}$ protein solutions in methanol/water/acetic acid (47.5/47.5/5%, v/v/v). In (a) multiply-protonated molecules of cytochrome c (MW 12 300) are recorded. The charge state distribution ranges from 13+ to 19+ while the base peak at m/z 767 corresponds to $[M+16H]^{16+}$. In (b) multiply protonated molecules of myoglobin (MW \sim 17 000) are recorded; the base peak at m/z 771 corresponds to $[M+22H]^{22+}$. The intensity of the signal for the multiply protonated molecule (or the ion formation efficiency) depends on the voltage applied to the source housing, with this dependence having almost the same form as that for the ion current shown in Fig. 1. On the other hand, the charge state distribution is almost independent of the applied voltage.

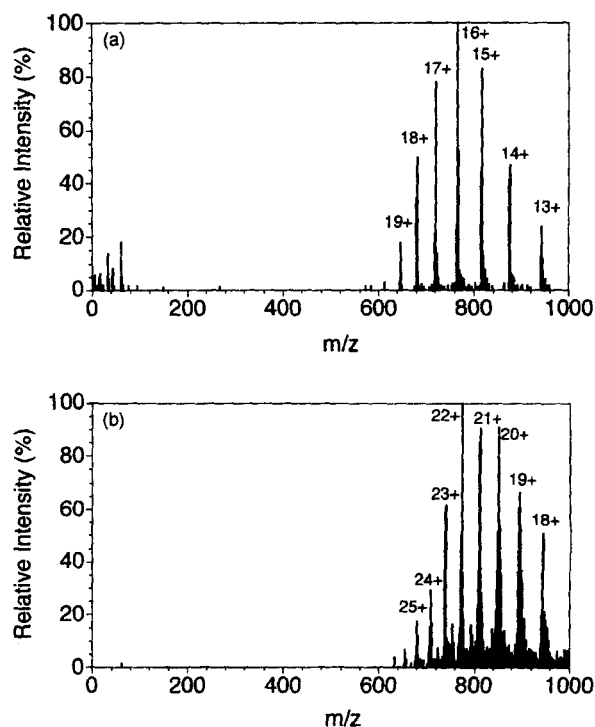


Figure 4. Mass spectra obtained from methanol/water/acetic acid (47.5/47.5/5.0%, v/v/v) solutions of (a) cytochrome c from sheep heart (MW 12 300) and (b) myoglobin from horse skeletal muscle (MW \sim 17 000). A high voltage of -1 kV was applied to the source housing and the gas-flow rate was 3.0 L/min.

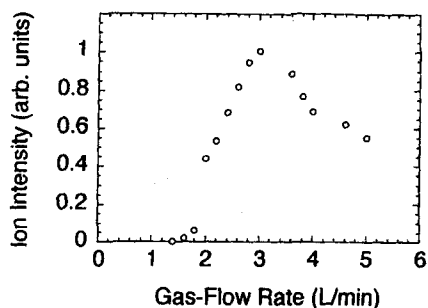


Figure 5. Gas-flow rate dependence of the intensity of the ion at $m/z=767$ ($[M+16H]^{16+}$) produced from a $1 \mu\text{M}$ solution of cytochrome c.

The details of ion formation from these charged droplets are not yet clearly understood. Evaporation of solvent molecules increases the charge density of a spray droplet to the Rayleigh limit, where the Coulomb repulsion becomes comparable with the surface tension. The resulting instability tears the droplet apart and produces charged daughter droplets that also evaporate. This sequence of events is repeated until the charge density of the droplet becomes so high that ions are desorbed from the droplet by the ion evaporation mechanism.⁷

At $V=0$ it is difficult to detect multiply protonated molecules in the mass spectra, as mentioned previously. This can be ascribed to the low charge density of the produced droplets. Since the diameter of the droplet initially produced is expected to be independent of the voltage, we conclude that the charge density of the droplet is increased with increasing voltage V .

Multiply-charged ions are also produced by electrospray and ionspray ionization techniques. In electrospray, a high voltage of 4~6 kV, relative to a counter electrode, is applied to a solution. Then, the solution forms a Taylor cone and the charged droplets are emitted from the cone tip towards the electrode by electric force.⁸ On the other hand, in pneumatically assisted electrospray (ionspray), the gas flow is used to enhance the evaporation of solvent molecules from charged droplets.⁹ The charge densities of the droplets produced by these techniques are likely to be similar to that produced by the present technique, since similar multiply charged ions are detected. In the present technique, however, charged droplets are produced from a solution by a shear stress from a sonic gas flow.

In Fig. 5, the intensity of the signal due to the multiply

protonated molecule of cytochrome c (m/z 767, $[M+16H]^{16+}$) produced from a $1 \mu\text{M}$ solution in methanol/water/acetic acid (47.5/47.5/5.0%, v/v/v) is shown as a function of the gas-flow rate. From the figure we conclude that the ion intensity has a maximum at about 3.0 L/min (sonic velocity). The gas-flow rate dependence of this ion intensity is quite different from that of the ion current shown in Fig. 3; in the supersonic region (>3.0 L/min) the ion intensity decreases whereas the total ion current remains almost unchanged. This can be ascribed mainly to the gas velocity dependence of the cross section of the gas flow, which has a minimum at sonic velocity.¹⁰ Since ions are introduced into the vacuum region from a sampling orifice with a diameter of 0.25 mm, the ion-transmission efficiency passing through the sampling orifice decreases with increasing cross section of the gas flow. Furthermore, at 1.0 L/min, for example, charged droplets are produced, as shown in Fig. 3, but no ions are detected. This can be ascribed to the formation of large droplets which are not suitable for ion formation, their charge densities are likely to be so low that ions remain in the droplets.

Acknowledgements

The authors thank Professors Y. Umezawa of the University of Tokyo and S. Kihara of Kyoto Institute of Technology for their fruitful discussions. They also acknowledge Drs K. Ogan and P. B. Grosshans for their invaluable assistance.

REFERENCES

1. A. Hirabayashi, M. Sakairi and H. Koizumi, *Anal. Chem.* **67**, 2878 (1995).
2. A. Hirabayashi, M. Sakairi and H. Koizumi, *Anal. Chem.* **66**, 4557 (1994).
3. (a) P. Delahay, *Double Layer and Electrode Kinetics*, Interscience Publishers, NY (1965). (b) J. N. Israelachvili, *Intermolecular and Surface Forces*, Academic Press, London (1985).
4. E. E. Dodd, *J. Appl. Phys.* **24**, 73 (1953).
5. M. L. Vestal and G. J. Fergusson, *Anal. Chem.* **57**, 2373 (1985).
6. J. V. Iribarne, *J. Rech. Atmosph.* **6**, 265 (1972).
7. J. V. Iribarne and B. A. Thomson, *J. Chem. Phys.* **64**, 2287 (1976).
8. J. B. Fenn, M. Menn, C. K. Meng, S. F. Wong and C. M. Whitehouse, *Science* **246**, 64 (1989).
9. A. P. Bruins, T. R. Covey and J. D. Henion, *Anal. Chem.* **59**, 2642 (1987).
10. H. W. Liepmann and A. Roshko, *Elements of Gasdynamics*, John Wiley & Sons, NY (1960).