Evaporation of Charged Droplets in Atmospheric Pressure Spray Mass Spectrometry

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Ions produced in atmospheric pressure spray (APS) ionization from aqueous solutions of sucrose and alanine are detected with a quadrupole mass spectrometer. The S/N ratios for their mass spectra are analyzed to study the evaporation of charged droplets, since they are detected as random noises. Two kinds of heated sampling orifices with thicknesses of 0.5 and 12.5 mm are compared. Higher ratios are obtained with the thicker one. The solution concentration dependence of the relative intensity of the cationized sucrose molecule suggests that ion evaporation does not take place in the sampling orifice and that the charged droplets produced by adiabatic expansion are suppressed by heating the vaporized solution. At the optimized pressures in the intermediate-pressure regions, 65 fmol of sucrose is detected. However, the noise level is still determined by detecting charged droplets, which are likely to be produced by the spray in the atmospheric-pressure region.

1. Introduction

Atmospheric pressure spray (APS) has extraordinary potential for analyzing of sugars, lipides, and peptides: Quasi-molecular ions of these nonvolatile molecules are produced at atmospheric pressure in a vapor jet of vaporized solution generated by a nozzle heated to about 400°C.^{1)~4)} In APS, the flow rates of the sample solutions are 0.5~2 ml/min, which typically coincide with the conventional high-pressure liquid-chromatography (HPLC) flow rates. Thus, APS is a suitable interface between a liquid chromatograph and a mass spectrometer (LC/MS). This situation is similar to atmospheric pressure chemical ionization (APCI) interface.⁵⁾

On the other hand, electrospray (ES) can produce highly charged quasi-molecular ions and is widely used, especially in analyzing proteins with high molecular weights.^{6), 7)} In pure ES, the flow rates of the sample solution are rather low, 1~5 μ l/min, which require significant postcolumn splitting or the use of either packed capillary columns or open tubular HPLC columns. However, recently, a high-flow ES (ion spray) interface for LC/MS with a flow rate up to 2 ml/min has been developed.⁸⁾ Thus, atmospheric pressure ionization methods such as APS, APCI, and ES are now available as practical LC/MS interfaces.

In these methods, the quasi-molecular ions are produced with high-density vaporized solution at atmospheric pressure and are introduced through a sampling orifice into an intermediate pressure region. Charged droplets or heavy ions might be formed in this region because of either clustering following adiabatic expansion, resulting from ion introduction into the vacuum region along with vaporized solution from the atmospheric pressure region through the sampling orifice, or because of the spray in the

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atmospheric pressure region. Since these charged droplets have much higher momentums than the quasi-molecular ions, the ion trajectories of the droplets in the vacuum region are rather difficult to control with electric and magnetic fields. Thus, when these droplets travel through the mass analyzing region of either a quadrupole mass spectrometer or a quadrupole ion trap, they are detected as major sources of random noises. This greatly reduces the sensitivity of the instrument. Until now, we have used a double-focusing mass spectrometer to study the production of quasi-molecular ions.^{2), 3)} In this type of mass spectrometer, however, no charged droplets are detected because the ion trajectory is curved.

In this work, by using a quadrupole mass spectrometer, the S/N ratio for the mass spectrum is analyzed in order to optimize the sensitivity of the instrument. In addition, the evaporation of charged droplets in both the sampling orifice and intermediate pressure regions is discussed. With respect to the signal level *S*, conventional computer programs, *e.g.*, SIMION,⁹⁾ are powerful tools for designing ion transmission systems for high-vacuum regions, but a system for intermediate-pressure regions ($P \geq 0.1$ Torr) is rather difficult to design because of collisional scattering. On the other hand, even when the signal level *S* is maximized experimentally, the S/N ratio is not necessarily optimized. Therefore, we have experimentally optimized the S/N ratio for the mass spectrum.

2. Experimental Setup

Figure 1 schematically illustrates our LC/MS system. The APS ion source is almost the same as the one described in our earlier work.²⁾ A deionized water solution was pumped at 1.0 ml/min into a nickel capillary (with an inner diameter of 0.1 mm) of the APS ion source. The heated region of the capillary was 30-mm long with heat generated mainly by infrared radiation from a Kanthal wire carrying about 15 A. A vapor jet was generated in the atmospheric pressure region, where neutral species and ions were produced. The distance between the capillary tip and the sampling aperture was 10 mm. There was no potential difference between the capillary and the orifice.

As shown in Fig. 1, the ions were introduced into the first intermediate pressure

Fig. 1. General schematic view of the APS/Q-Mass system. P_1 and P_2 represent the pressures in the first and second intermediate-pressure regions.

region through a sampling orifice and then into the second one through the first aperture in the electrode. The sampling orifice was heated with a ceramic heater (50 W) to about 120°C. Remaining gases in the first intermediate pressure region were pumped through the second intermediate pressure region, which was then evacuated by a 600-l/s turbo molecular pump and a 1000-l/min rotary pump.

The pressure P_2 in the second intermediate pressure region was varied between 0.1 ~1.0 Torr using a vacuum valve while the pressure P_1 in the first intermediate pressure region was adjusted by opening and closing the eight screw holes in the electrode. ($P_1 = 0.5 \sim 6$ torr, $P_1 \geq P_2$) The pressures P_1 and P_2 were measured with Pirani vacuum gauges (ULVAC WP-02). A drift voltage of ΔV_1 was applied between the sampling orifice and the first aperture, and ΔV_2 was applied between the first and second apertures. These voltages were applied to dissociate cluster ions by inducing collisions and to attain high ion-transmission efficiency.

A quadrupole mass analyzer (Balzers) was operated at 7×10^{-7} Torr. The mass-analyzed ions were accelerated by a post-acceleration dynode, to which we applied a high voltage of 5 kV. Collisions between the ions and the dynode produced electrons and ions from its surface. These particles were detected by an electron multiplier and the resulting output was fed into a digitizing oscilloscope (HP 54111D).

3. Sampling Orifice

Charged droplets are produced by the spray when the temperature of the capillary is below 400°C.⁴⁾ The charged droplets are also produced by an adiabatic expansion caused when the ions in a higher-pressure region are introduced along with vaporized solution into a lower-pressure region. Many charged droplets are produced by adiabatic expansion in LC/MS interfaces, such as APS, with a flow rate of 1 ml/min. This is because the density of water molecule in the vapor jet is estimated to be about 10^{25} m^{-3} , which is one order of magnitude higher than that of air with a humidity of 100% at 20°C. Since ions in the intermediate-pressure regions are accelerated by the drift voltage, ions hydrated with less than four water molecules can be decomposed by collisions.¹⁰⁾ Contrarily, charged droplets with huge numbers of water molecules are difficult to evaporate in the intermediate-pressure regions. This leaves only the structure of the sampling orifice and the pressures in the intermediate-pressure regions as parameters.

3.1 Thickness of sampling orifice

A comparison of S/N ratios for the spectrum has been made between two kinds of sampling orifices. One has an aperture with an inner diameter of 0.3 mm and a thickness of 0.5 mm. This orifice is similar to that used in our previous experiments.^{2), 3)} The other has an inner diameter of 0.25 mm and a thickness of 12.5 mm. This kind of orifice is often used in electrospray interfaces.¹¹⁾ In the first intermediate-pressure region, the difference in the distance the ion must travel from the orifice to the first aperture, as determined by the relative orifice thickness, is negligible.

Mass spectra from an aqueous solution of alanine (1 μM) have been obtained and the S/N ratio for the spectrum of the protonated alanine molecule MH^+ ($m/z = 90$) has been analyzed as a function of ΔV_1 for each combination of P_1 and P_2 , while ΔV_2 is fixed at 10

Fig. 2. S/N ratio dependence on drift voltage ΔV_1 for the spectra of protonated alanine (○) and the fragment ion (●) produced from an aqueous solution of alanine ($1 \mu\text{M}$). The error bars show statistical error. The sampling orifice has an aperture with a thickness of (a) 0.5 mm and (b) 12.5 mm. $\Delta V_1 = 10 \text{ V}$.

Fig. 3. Relative intensity of the cationized sucrose shown as a function of the sucrose concentration of the aqueous solution. The error bars show statistical error. The solid line has a slope of 1.3.

V. Fig. 2 shows typical examples at $P_1 = 1.6 \text{ Torr}$ and $P_2 = 0.5 \text{ Torr}$. With increasing ΔV_1 , the S/N ratio of the fragment ion ($m/z = 44$) increases. This trend indicates that the fragment ions are produced by collisions of MH^+ with neutral species in the first intermediate-pressure region. In Fig. 2(a), the S/N ratios of MH^+ and the fragment ion are similar at $\Delta V_1 \sim 50 \text{ V}$, whereas in (b) the corresponding crossing point is at $\Delta V_1 \sim 70 \text{ V}$, which is rather high. This difference in the drift voltage at the crossing point can be ascribed to the following reason: Since, in the orifice with a thickness of 12.5 mm, the droplets are heated more effectively than in the orifice with a thickness of 0.5 mm, the pressure around the ion trajectory in the intermediate-pressure region is higher than that monitored. Thus, less fragment ions are produced by collisions because of a lower collision energy. It should be noted that the fragment ions are not produced by the pyrolysis of MH^+ in the heated orifice as verified by the lack of fragment-ion detection at $\Delta V_1 = 20 \text{ V}$.

In Fig. 2(a), the S/N ratio for MH^+ has a maximum of about 1000 at $\Delta V_1 = 40 \text{ V}$, while in (b), it is about 2000 at $\Delta V_1 = 60 \text{ V}$. Therefore, it is concluded that in the thick orifice the maximum value of the S/N ratio is higher than that in the thin orifice. For other combinations of P_1 and P_2 , it is found that the optimized S/N ratios in the thick

orifice are always higher than those in the thin orifice.

In the thick orifice, the recombination of ions with the metal surface of the orifice is negligible. This is because the ion currents detected with the first electrode have been nearly equal in both orifices. This shows that the currents of ions passing through the orifices are nearly equal. Thus, a laminar flow of the vaporized solution is likely to be generated within the orifice.

3.2 Ion production in the sampling orifice

In the following discussion, we consider the evaporation of charged droplets in the 12.5 mm thick sampling orifice. Fig. 3 shows the relative intensity of MNa^+ produced from an aqueous solution of sucrose containing $0.4 \mu\text{M}$ NaOH. It is shown as a function of the sucrose concentration in a log-log plot: The total ion intensity is taken as 100%. If the ion were produced solely by an ion-molecule reaction (cation-attachment reaction) in the gas phase, the relative intensity should be proportional to the concentration of the solution. However, for the results obtained at 440°C , the slope of the solid line is 1.3, which is significantly higher than 1.0. Thus, we conclude that the cationized sucrose molecule is produced by ion evaporation as well as by ion-molecule reactions in the gas phase and that the 1.3 slope is determined by a sum of the contributions of each process. In Fig. 3, the relative intensities obtained at 410°C are lower than those at 440°C , but have a similar concentration dependence. This systematic deviation is ascribed to the increase in the noise level caused by the charged droplets.

In the sampling orifice, the contribution of the gas-phase ion-molecule reaction is expected to be negligible in comparison with that in the atmospheric pressure region. This is because 1) the gas density is significantly lower in the sampling orifice than in atmospheric pressure and 2) the travel time of ions passing through the orifice is expected to be shorter than that in the atmospheric-pressure region, since the travel distance is nearly equal. On the other hand, the contribution of ion evaporation might be higher in the orifice if charged droplets are produced in the atmospheric-pressure region because the vaporized solution is appreciably heated there, as described in the previous section. However, we noted in our previous experiment²⁾ the same concentration dependence of the relative intensity when the sampling orifice has a 0.2 mm thick aperture as shown in Fig. 3. The contribution of ion evaporation in the sampling orifice is expected to be much lower than that in the present one, since the travel time of charged droplets passing through the orifice is much shorter and the vaporized solution is heated to a lesser degree. Therefore, the contribution of ion evaporation from charged droplets in the present sampling orifice is negligible, even though charged droplets, if any, are appreciably heated. This shows that, since the vaporized solution is heated in the sampling orifice the production of charged droplets by adiabatic expansion is suppressed when ions are introduced into the first intermediate-pressure region.

4. Pressures in the Intermediate-Pressure Regions

The S/N ratio of the mass spectra related to the thick orifice has been analyzed while changing P_1 and P_2 . For quasi-molecular ions, the signal level is low when P_1 and P_2 are low, because these ions are significantly accelerated by drift voltages and dissociated by collisions. When P_1 and P_2 are so high that there is significant collisional

scattering, the signal level is low since efficiency of ion transmission through the second and third apertures by the drift voltages decreases. Thus, the S/N ratios of the mass spectra are expected to be optimized under some combination of P_1 and P_2 .

Fig. 4 shows S/N ratio dependence on P_1 and P_2 for the spectra of the quasi-molecular ions of alanine and sucrose: The protonated alanine molecule ($\circ$, $m/z=90$) is produced from an aqueous solution of alanine ($1\text{ }\mu\text{M}$) and the cationized sucrose molecule ($\triangle$, $m/z=365$) is from an aqueous solution of sucrose ($10\text{ }\mu\text{M}$) and NaOH ($0.4\text{ }\mu\text{M}$). Results are obtained for (a) $0.1\text{ Torr} \leq P_1 \leq 0.5\text{ Torr}$ ($P_1=P_2$; the first aperture is removed), (b) $0.5\text{ Torr} \leq P_1 \leq 0.7\text{ Torr}$, and (c) $0.85\text{ Torr} \leq P_1 \leq 0.95\text{ Torr}$, where P_2 varies from 0.1 to 0.5 Torr. The results shown in these figures are obtained at the optimized drift voltages of ΔV_1 and ΔV_2 , a typical example of which is shown in Fig. 2.

In Fig. 4, all S/N ratios other than that for alanine, shown in (a), decrease with increasing pressure. This decrease results from the increase in collisional scattering. In

Fig. 4. S/N ratio dependence on P_1 and P_2 for the spectra of protonated alanine ($\circ$) produced from a $1\text{-}\mu\text{M}$ aqueous solution and cationized sucrose ($\triangle$) from a $10\text{-}\mu\text{M}$ solution. The error bars show statistical error.

Fig. 5. S/N ratio for the spectrum of cationized sucrose produced from an aqueous solution of sucrose ($10\ \mu\text{M}$) shown as a function of temperature at the capillary tip of the APS ion source. The error bars show statistical error.

Fig. 6. Mass chromatogram of 65 fmol of sucrose obtained in a SIM mode.

Fig. 4(a) the S/N ratio for alanine at 0.5 Torr is higher than that at 0.1 Torr. This is because, at lower pressure, many fragment ions (not shown in this figure) are produced by the collisions. In (a), the S/N ratios are below 2000. In (b), the S/N ratios peak at about 2000 when $P_1=0.5$ Torr and $P_2=0.1$ Torr. In (c), the S/N ratio for the protonated alanine molecule peaks at about 4000 when $P_2=0.1$ Torr, while the peak for the cationized sucrose molecule is rather low, at about 1000. Thus, we conclude that the optimized pressures depend on the ionic species. Since the concentration of the sucrose solution is higher by a factor of 10 than that of the alanine solution, we conclude that the optimized pressures for P_1 and P_2 are 0.5 and 0.1 Torr, respectively.

In (a), at $P_1=P_2=0.5$ Torr, the cationized sucrose molecule is not detected. Since fragment ions are detected at a high drift voltage ΔV_1 (not shown in the figure), we know that the transmission efficiency of the cationized sucrose molecule is greatly decreased by collisional scattering. On the other hand, the protonated alanine molecule is detected with a high S/N ratio at the same pressures. This is ascribed to the difference in the collisional scattering rates and the dissociation energies of the ions.

At $P_1=0.5$ Torr, the ratios shown in (a) are lower than those in (b), although the total ion travel distance is the same. This can be attributed to a high ion-transmission efficiency at the lower pressure and to the suppression of charged-droplets formation. When the ratio of upstream to downstream pressures is low, fewer charged droplets are produced by adiabatic expansion. This is because the length of the supersonic jet following adiabatic expansion is given by the pressure ratio. The gas temperature, which has a minimum at the Mach disk (the end of the supersonic jet range), increases with decreasing jet length.¹²⁾

5. Detection Limit

When the temperature of the ion-source capillary is below 400°C, mass spectra cannot be obtained in instruments with an aperture orifice such as the one we used previously.^{2), 3)} In the present instrument, which uses a thick orifice, the mass spectra are obtained at capillary temperatures as low as 320°C. Fig. 5 shows the S/N ratio of the spectrum for the cationized sucrose from an aqueous solution of sucrose (10 μ M) and NaOH (0.4 μ M). It is shown here as a function of the temperature of the ion-source capillary. As the temperature increases, the S/N ratio increases and saturates at temperatures above 430°C. The low S/N ratio at low temperatures can be attributed to the charged droplets formed by the spray produced under atmospheric pressure.

Fig. 6 shows a mass chromatograph of the cationized sucrose molecule obtained in a single ion monitor (SIM) mode: 65 fmol of sucrose is detected with a S/N ratio of about 10. This value is half as large as that previously reported when using the double-focusing mass spectrometer,¹⁾ in which charged droplets cannot be detected as random noise. It should be noted that the observed noise level is still one order of magnitude higher than the level caused by the dark current of the electron multiplier. The droplets detected are most likely produced by the spray produced under atmospheric pressure.

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Keywords

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