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ABSTRACT

The author has developed techniques to couple a liquid separation tool, e.g. a semi-micro liquid chromatograph (LC), a conventional liquid chromatograph, or capillary electrophoresis (CE), to a mass spectrometer by using sonic spray ionization (SSI), which was developed by the author's group, as an interface. The efficiency of negative-ion formation in sonic spray ionization was increased by adding three percent of ammonia to a sample solution. In SSI, a sample solution is sprayed from a sample-introduction capillary with a high-speed gas flow coaxial to the capillary and ions of compounds in the solution are produced at room temperature and atmospheric pressure. Since various kinds of organic compounds, e.g. thermolabile, volatile, and nonvolatile compounds, are readily ionized, many kinds of compounds in a mixture can be simultaneously analyzed with the SSI interface. Furthermore, solutions containing phosphate buffers, which are difficult to use in other ionization techniques, can be used in SSI because high-conductivity solutions are stably sprayed using only the gas flow. However, since the solution flow rates in SSI initially ranged from 10 to 100 μL/min, they did not match the range of solution flow rates of commonly used separation tools (conventional LC: 1000 μL/min or higher, semi-micro LC: 100 to 200 μL/min, CE: $\sim$0.1 μL/min). Therefore, it was difficult to use SSI as an interface between these separation tools and a mass spectrometer. Furthermore, the production of negative ions through SSI had not been confirmed. In this study, the author has confirmed that negative ions can be produced by SSI, and has developed techniques to

enable the use of sonic spray ionization to couple a separation tool with a mass spectrometer.

The production of negatively charged droplets in SSI was previously confirmed by measuring the ion current of the sprayed gas. However, when the author measured negative ions with a mass spectrometer under the same solution conditions as for positive ion analysis, no intense analyte ions were obtained. Therefore, the author sought to determine what conditions were suitable for negative ion formation in SSI, and found that the efficiency of negative ion formation was increased by adding three percent of ammonia to the sample solution. This enabled the analysis of negative ions without discharge occurring.

The author has also found that ions were produced in a sprayed gas containing a high density of gas-phase solvent molecules at the solution-flow rates of semi-micro and conventional LC. However, these ions associated with solvent molecules and became large clusters and droplets due to adiabatic expansion when they were introduced into the vacuum region through the sampling orifice of a mass spectrometer. This reduced the sensitivity of the mass spectrometer. Therefore, the author has developed a technique to reduce the density of the gas-phase solvent molecules in the spray which prevents the association of ions and solvent molecules. For a semi-micro liquid chromatograph / mass spectrometer (LC/MS), the density of solvent molecules in the spray was reduced by increasing the gas-flow rate used to spray the solution. In this case, a gas flow rate of 6 L/min was needed for a solution flowing at 200 μL/min. For conventional LC/MS, in which the solution flow rate is much higher, the author has developed a multi-hole plate, which does not have a hole coaxial to the sampling orifice but has small holes around the central region of the plate. The solvent density was reduced by diffusing the sprayed gas

with the multi-hole plate, then the sprayed gas was introduced into the vacuum region of the mass spectrometer. Using these techniques, SSI can be used at solution-flow rates ranging from 10 to 1000 μL/min, and a semi-micro and a conventional LC can be directly coupled with a mass spectrometer through the SSI interface.

When SSI was used as an interface for a capillary electrophoresis / mass spectrometer (CE/MS), in which the solution flow rate is much lower, another problem arose. In SSI, the pressure around the tip of the sample-introduction capillary is reduced by the high-speed gas flow nebulizing the solution, so the solution was pumped into the capillary at a flow rate of about 0.1 μL/min due to the pressure difference between the two ends of the capillary. Though this pumping rate did not affect the LC separation, it was excessive for CE separation because the solution-flow rate in CE is below 0.1 μL/min. Thus, this pumping was likely to lower the resolution of CE separation. To avoid this, the author developed a CE/MS interface where a buffer reservoir was added between the sample-introduction capillary of the interface and the electrophoresis capillary. This prevented the solution in the electrophoresis capillary being pumped because the solution in the buffer reservoir was pumped into the sample-introduction capillary by the pressure difference. The author has also demonstrated CE/MS analysis with a mobile-phase buffer containing phosphates by filling the buffer reservoir with an acetic-acid solution as a substitute for the mobile-phase buffer. This increased the ion intensity one-hundred fold. This SSI interface enabled high-sensitivity analysis even when using a phosphate buffer, whose use has been generally avoided in CE/MS.

The techniques developed in this study enable us to analyze various kinds of liquid mixtures under virtually any normal conditions. Therefore, LC/MS and CE/MS using SSI are expected to become powerful tools in various fields of analysis.

CONTENTS

ABBREVIATIONS

API	: Atmospheric Pressure Ionization
APCI	: Atmospheric Pressure Chemical Ionization
APS	: Atmospheric Pressure Spray
CE	: Capillary Electrophoresis
CE/MS	: Capillary Electrophoresis / Mass Spectrometry (Spectrometer)
CF-FAB	: Continuous-flow Fast-atom Bombardment
ESI	: Electrospray Ionization
Frit-FAB	: Frit Fast-atom Bombardment
GC	: Gas Chromatography (Chromatograph)
GC/MS	: Gas Chromatography / Mass Spectrometry (Gas Chromatograph / Mass Spectrometer)
IS	: Ion Spray
LC	: Liquid Chromatography (Chromatograph)
LC/MS	: Liquid Chromatography / Mass Spectrometry (Liquid Chromatograph / Mass Spectrometer)
MS	: Mass Spectrometry (Spectrometer)
SSI	: Sonic Spray Ionization

1. Introduction

1.1 Coupling of Chromatography with Mass Spectrometry

Chromatography is an important analytical method used to separate complex mixtures. Chromatographic techniques are divided into two types, gas chromatography and liquid chromatography, in which the mobile-phases are gases and liquids, respectively. There are several forms of liquid chromatography; e.g., liquid chromatography (LC) in which packing columns are used, thin layer chromatography, high-speed countercurrent chromatography, and supercritical fluid chromatography. Another commonly used liquid-separation method is capillary electrophoresis, in which ionic substances are separated in a capillary column by electrophoresis. The substances separated by liquid-separation tools are generally detected by optical detectors, e.g., ultraviolet and fluorescent spectrophotometers. However, certain species of substances cannot be identified by these optical detectors.

Mass spectrometry is an analytical technique used to identify species of substances by measuring the mass-to-charge ratio (m/z) of molecular ions. In this method, an electric

field or a magnetic field is applied to the gaseous-molecular ions in a vacuum region to enable mass separation. Mass spectrometry is a highly sensitive detection technique. Therefore, the coupling of chromatography and mass spectrometry, i.e. chromatography / mass spectrometry, is becoming a powerful analytical technique used to analyze mixtures.

Liquid chromatography / mass spectrometry (LC/MS) is the most commonly used technique, because the range of LC applications is the widest in the field of chromatography. Capillary electrophoresis / mass spectrometry (CE/MS) is also expected to be widely used, though, because CE has a higher separation resolution than LC [1].

1.2 Interface Techniques for Liquid Chromatography / Mass Spectrometry

A chromatograph / mass spectrometer is divided into three parts, a chromatograph to separate a mixture, a mass spectrometer to detect sample ions, and an interface to couple the chromatograph and mass spectrometer. In developing a better chromatograph / mass spectrometer, the ionization method used as the interface between the chromatograph and the mass spectrometer is one of the most important techniques. Development of an interface for a liquid-phase separation tool and a mass spectrometer, such as LC/MS and CE/MS, is especially difficult, because sample molecules dissolved in a solution have to be converted into gaseous ions. Various types of interfaces for LC/MS and CE/MS have been

proposed.

The LC/MS and CE/MS interfaces are roughly divided into two types. In one, ions are produced in a vacuum region. In the other, ions are produced at atmosphere pressure then introduced into the vacuum region. In the early stages of the development of LC/MS, the belt conveyor method were proposed. In this method, samples eluted from the LC were dropped onto the belt conveyor, heated to evaporate the solvent, then introduced into the vacuum region. Ions were then produced by electron impact in the vacuum region [2,3]. After that, thermospray [4-6] and continuous flow fast-atom bombardment (CF-FAB [7,8]) interface techniques were developed. In thermospray, ions were produced by spraying the sample solution into a vacuum region directly by using a heated capillary [4-6]. In CF-FAB, ions were produced in a vacuum region by applying an atom beam to the sample solution eluted from the inlet capillary into the vacuum region [7,8]. The frit-FAB technique [9,10], in which a small metallic frit is set at the end of the inlet capillary of the FAB interface, was also developed.

Recently, atmospheric pressure ionization (API), in which ions are produced at atmosphere pressure and introduced into a vacuum region, has become important. In API, the solvent is evaporated in the atmosphere, so the inner walls of the mass spectrometer's vacuum region are less likely to be contaminated.

1.3 Atmospheric Pressure Ionization

In atmospheric pressure ionization (API), charged droplets, i.e. sample molecules and ions enclosed by solvent molecules and charges, are formed from the sample solution. Then quasi-molecular ions of the sample molecules are produced from the charged droplets by evaporating the solvent. The quasi-molecular ions are molecules combined with cations or anions, e.g. protons, sodium ions and chlorine ions, and deprotonated molecules. There are two types of techniques used to produce charged droplets: applying heat to a capillary in which a solution flows to nebulize the solution, and applying a high voltage to a metallic capillary to electrostatically nebulize the solution in the capillary. The former produces an atmospheric pressure spray (APS) [11,12] and atmospheric pressure chemical ionization (APCI) [13,14]. In APS, ions are produced only when the solution is nebulized by heat. In APCI, charged droplets are produced with a heat nebulizer, then ions are produced by applying a corona discharge to the evaporated gaseous sample molecules from the charged droplets. The corona discharge initiates ion-molecule reactions, e.g. proton transfer, and ionizes sample molecules:

$$M + AH^+ \rightarrow (M+H)^+ + A \qquad (1.1)$$

$$M + B^- \rightarrow (M-H)^- + B \qquad (1.2)$$

The chemical reaction in equation (1.1) is initiated by applying a positive corona discharge to the gaseous sample molecule. When the proton affinity of sample molecule M is stronger than that of molecule A, the positive sample ion $(M+H)^+$ (a protonated molecule)

is produced. Generally, the proton provider AH^+ is an H_3O^+ ion. The other hand, the chemical reaction in equation (1.2) is initiated by applying a negative corona discharge. When the proton affinity of sample molecule M is weaker than that of molecule B, the negative sample ion $(M-H)^-$ (a deprotonated molecule) is produced. Generally, B^- is an OH^- ion [15].

When a high voltage is used to nebulize the solution, electrospray ionization (ESI) [16-18] and ion spray (IS) [19] are produced. The ion spray technique is also called pneumatically assisted ESI, in which an assisted nebulizing gas flows around the metallic capillary to enhance evaporation of the charged droplets. In ESI and IS, the solution is nebulized by applying a high voltage between a metallic capillary in which a solution flows and a counter electrode located a few millimeters away from the capillary tip. In this case, the emerging liquid assumes an equilibrium conical shape (a Taylor cone) with a sharp tip from which flows a stream of charged droplets ejected by electrostatic force. A partial separation of the positive and negative ions present in the solution occurs near the capillary tip and the electrosprayed droplets therefore contain excess ions of one charge. Positively and negatively charged droplets are produced by applying high positive and negative voltages (3-4 kV) to the metallic capillaries, respectively. The charged droplets are evaporated, then positive and negative ions ($(M+H)^+$ and $(M-H)^-$) are produced from the positively and negatively charged droplets, respectively.

In ESI and IS, there is general agreement that these charged droplets evaporate until the increased surface charge density confers instability. At this stage (the Rayleigh limit) the electrical forces due to the surface charge approach equality with those due to surface tension and a droplet disintegrates into several much smaller charged droplets. The maximum permissible charge at the Rayleigh limit, q_r, is given by the equation

$$q_r = (8\pi/e)(\gamma \epsilon_0)^{1/2} r^{3/2} \qquad (1.3)$$

where r is the droplet radius, γ is the surface tension, and ϵ_0 is the permittivity of free space. A succession of such fission processes, yielding smaller and smaller droplets, occurs as evaporation continues, then gas-phase analyte ions are formed from the charged droplets. In ESI and IS, analyte ions already present in the solution are transferred to the gas-phase; this occurs either because the analyte is ionic or because it is associated with other ions present in the solution, e.g. by protonation.

While there is general agreement on the mechanism for the initial stages of charged droplet subdivision, different ideas have been used to explain the eventual formation of analyte ions from the liquid phase. According to one mechanism, the division of charged droplets at the Rayleigh limit is repeated until the droplets finally contain a single analyte molecule. This analyte molecule would eventually be observed as a gas-phase ion provided that a charge was retained by the analyte rather than being lost with evaporating solvent molecules. A more appealing mechanism for the formation of gas-phase ions is field desorption. According to this mechanism, the electric field at the surface of the charged droplet increases with the decreasing radius as the solvent evaporates. An analyte molecule that has accumulated sufficient charge is then able to evaporate or desorb from the charged droplet, alone or in association with one or more molecules of solvent [15].

Furthermore, ESI and IS can produce multiply-charged ions of large molecules with over three charges. Multiple charging in ESI and IS creates a whole series of molecular ion peaks each of which represents a different charge state for the same compound. The molecular weight may be determined from such a spectrum if it is assumed, as is invariably

the case, that adjacent peaks in the series differ by only one charge. If two adjacent peaks are selected from this series, then for an ion of measured mass-to-charge ratio (m/z), m_i, which originates from an analyte with molecular weight M_R and carries an unknown number of charges, i, supplied by proton attachment, m_i and M_R are related by

$$m_i = (M_R + iM_H) / i \qquad (1.4)$$

where M_H is the mass of a proton. The mass-to-charge ratio, m_{i+1}, for an ion that carries one additional proton attached to the same analyte molecule is then given by

$$m_{i+1} = (M_R + (i+1)M_H) / (i+1) \qquad (1.5)$$

so that i, the charge state, may be determined from the two m/z values using

$$i = (m_{i+1} - M_H) / (m_i - m_{i+1}) \doteqdot (m_{i+1} - 1) / (m_i - m_{i+1}) \qquad (1.6)$$

Once the charge state has been calculated in this way, each peak in the series can be used to provide a separate estimate of the molecular weight, M_R [15].

Of the API techniques, APCI, ESI, and IS are the most widely used for LC/MS and CE/MS, but the proper use of these techniques depends on the type of sample compounds. Although APCI is useful for analyzing volatile and thermostable compounds, it cannot be used for nonvolatile and thermolabile compounds. On the other hand, ESI and IS are available for the analysis of nonvolatile and thermolabile compounds. Furthermore, ESI and IS can produce multiply-charged ions of large biomolecules such as proteins. Since a

measurement range of the m/z value in most types of mass spectrometer are limited, for example, the range of the m/z value in a quadrupole and ion trap mass spectrometers ranges from 0 to 2000, singly-charged large biomolecules with a molecular weight of over 2000 cannot be measured. Therefore, the production of multiply-charged ions is important, and these techniques enable the use of mass spectrometry in the field of bioscience.

However, the sprays of high-conductivity solutions are unstable in ESI and IS, so solutions containing highly concentrated electrolytic compounds cannot be used with these techniques. Therefore, the range of solutions that can be used in ESI and IS are limited. For example, nonvolatile buffers, such as phosphate, which are generally used in CE, are difficult to use in ESI and IS. Furthermore, when a higher negative voltage is applied to a metallic capillary used to produce negative ions, undesirable discharges may occur due to electron emission from the tip of the capillary. These discharges may interfere with the ion formation in ESI and IS.

Which API techniques can be properly used also depends on the solution-flow rates of the liquid separation tools. LC is divided into three types depending on the solution-flow rates: conventional LC (1000 μL/min or over), semi-micro LC (100-200 μL/min), and micro LC (several μL/min). In CE, the solution-flow rates are below 0.1 μL/min. APCI is used for conventional and semi-micro LC/MS, but cannot be used with low solution-flow rates, i.e. those of micro LC and CE. ESI and IS are used for the liquid separation tools that have low solution-flow rates, i.e. semi-micro LC, micro LC, and CE. Though IS can also be used at high solution-flow rates (i.e. above 1 mL/min), multiply-charged ions cannot be produced at such solution-flow rates.

Thus, the interface between the liquid-separation tool and mass spectrometer has to be changed depending on the samples, the solution used, and the solution-flow rate. When we

want to analyze various samples using different solutions, we need several types of interface and changing the interface takes a considerable amount of time and effort. Therefore, an interface that can be used over a wide range of solution-flow rates, samples, and solutions, would be a significant step forward.

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2. Sonic Spray Ionization

2.1 Characteristics of Sonic Spray Ionization

Recently, the author's group developed the sonic spray ionization (SSI) technique [1,2]. In this technique, a sample solution is sprayed from a fused-silica sample-introduction capillary by a high-speed nitrogen gas flow that is coaxial to the capillary, and singly and doubly-charged ions of compounds in the solution as well as charged droplets are produced at room temperature and atmospheric pressure (Fig. 2.1). In SSI, the ion intensity depends on the gas velocity as shown in Fig. 2.2. The ion intensity reached a maximum at the gas flow rate of 3 L/min. At this flow rate, the gas was moving at close to the sonic velocity. The reason for the maximum ion intensity occurring at sonic velocity is likely to be as follows. Generally, the ionization efficiency of API depends on the initial size of the charged droplets. The ionization efficiency increases as the initial size of charged droplets decreases because fine droplets evaporate more quickly. In SSI, the size of the charged droplets decreases as the gas velocity increases. Thus, the ionization efficiency of SSI increases as the gas velocity increases. However, since shock waves are generated in the sprayed gas in the supersonic region, the fine droplets become associated with each other

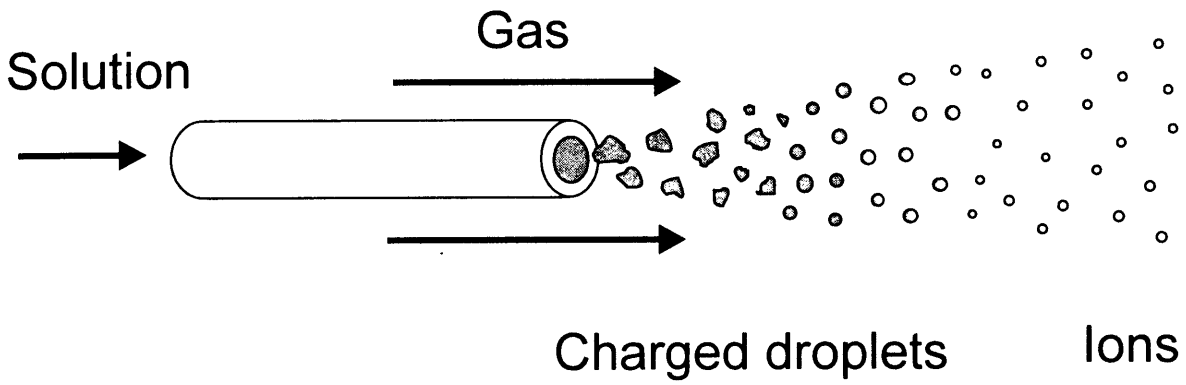

Figure 2.1 Schematic diagram of sonic spray ionization. A solution is sprayed with a high-speed gas flow, and ions are produced under atmospheric pressure.

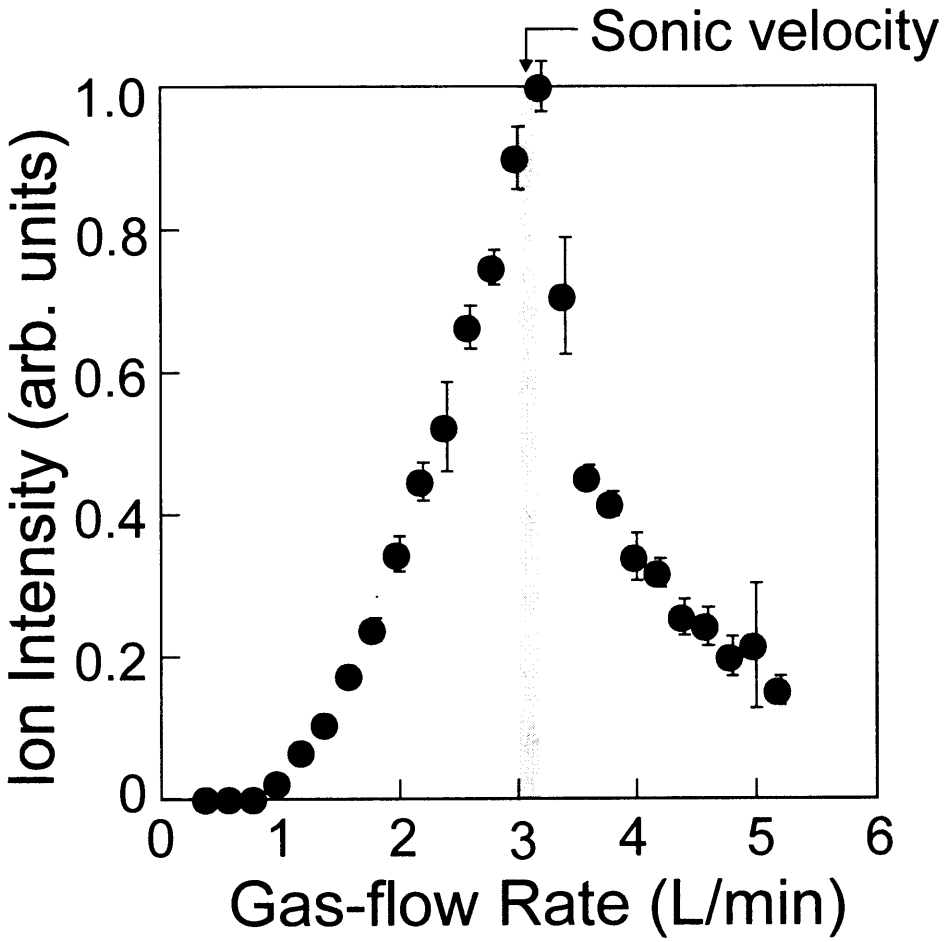

Figure 2.2 Ion intensity of doubly-protonated gramicidin-S molecules (m/z = 571) from a 1-μM solution as a function of the gas-flow rate. The solution flow rate was 30 μL/min.

and form large droplets. Therefore, the ion intensity in SSI decreases within the supersonic region as shown in Fig. 2.2.

In SSI, the origin of the charged species is ascribed to the non-uniformity of the ion concentrations at the surface of the droplets. Figure 2.3 is a schematic diagram of the ion formation in SSI. Since the solution in the capillary is held at ground potential, droplets produced with a high-speed gas flow are electrically neutral in total. An electrical double layer is then formed on the surface of the droplet by the phase boundary potential between the gas and the solution; for example, when the solution is made from pure water, a negatively charged species gathers on the surface and a positively charged species gathers under the layer of the negative charged species. The depth of the double layer is of the order of 10 nm, although this depends on the ion concentration [3]. When such a surface is disrupted by a shear stress due to the sonic gas flow, charge separation occurs and charged droplets are produced. Since the amounts of positively and negatively charged species in the sprayed gas are the same, the sprayed gas of SSI is electrically neutral. The size of the charged droplets is decreased by fission due to the Rayleigh disintegration and positive and negative ions are produced from the fine charged droplets through the evaporation of solvents or the desorption of analyte ions; this is the same mechanism as in ESI and IS [section 1.3]. However, although the formation of positive ions was earlier confirmed by mass spectrometry, the formation of negative ions had not been confirmed at this point.

The charged droplets initially produced in SSI are very small, i.e. about 1 μm in diameter [4]. This is smaller than the droplets produced by other API techniques, e.g. about 10 μm in ESI [5]. Thus, the ionization efficiency of SSI is higher than that of the other techniques. The higher ionization efficiency can be seen, for example, in the analysis of catecholamines [2]. Figure 2.4(a) and (b) show mass spectra obtained from a 10-nM

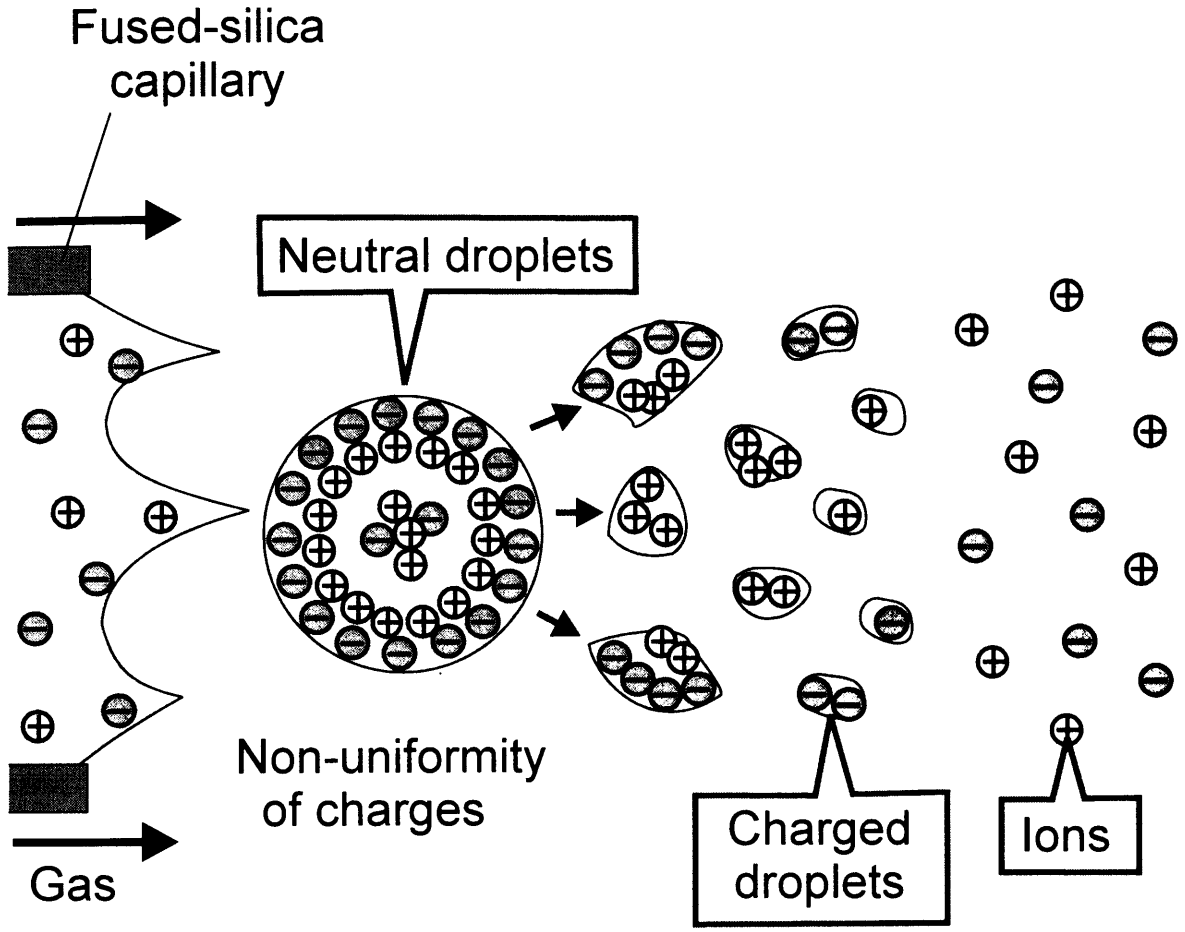

Figure 2.3 Schematic diagram of ion formation in the sonic spray. The origin of the charged species is ascribed to non-uniformity in the positive and negative ion concentrations at the surface of the droplets. Charged droplets are produced by the fission of the droplets with the sonic gas flow. Ions are likely produced from the charged droplets by the evaporation of solvents.

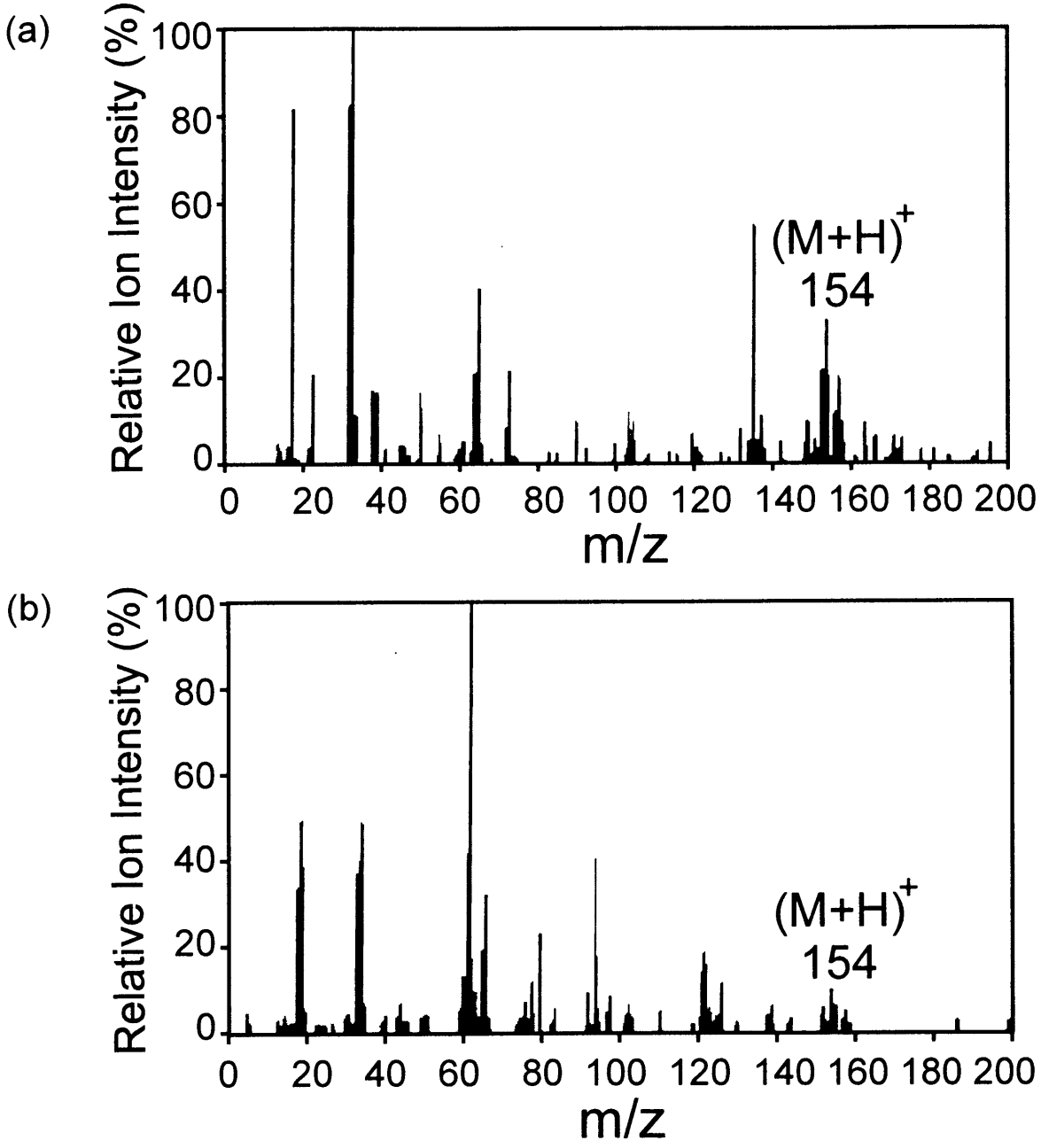

Figure 2.4 Mass spectra obtained from a dopamine solution (methanol/water = 50/50%, v/v) by (a) sonic spray ionization, and (b) ion spray ionization, at a solution flow rate of 30 μL/min. The solution concentrations were (a) 10 nM, and (b) 1 μM.

dopamine solution by SSI and a 1-μM dopamine solution by IS, respectively. Though the protonated dopamine molecules (m/z = 154) were observed in both spectra, the ion intensity of the dopamine analyzed by SSI was one-hundred times as high as that by IS. Also, thermolabile compounds are readily analyzed with SSI, because heat does not have to be applied to the interface to produce ions. Both volatile and nonvolatile compounds can be analyzed by SSI. Furthermore, SSI can be used with a wide range of buffer solutions regardless of the conductivity of the solutions, because the SSI sprays are generated only by gas flow.

2.2 Formation of Multiply Charged Ions by Sonic Spray Ionization

When a voltage is applied to a metallic source housing set around the fused-silica capillary, an electric field is applied to the solution in the capillary, and non-uniformity of the charge density arises at the solution surface in the capillary. (The solution was isolated from the source housing by the capillary.) In this case, species with a charge opposite to that of the applied voltage were gathered at the solution surface due to the electric field caused by the voltage, as shown in Fig. 2.5. Since the solution was grounded, charged species with the same polarity as the voltage flowed out through the earth electrode as current. Thus, the charge density of the droplets increases and the sprayed gas is charged.

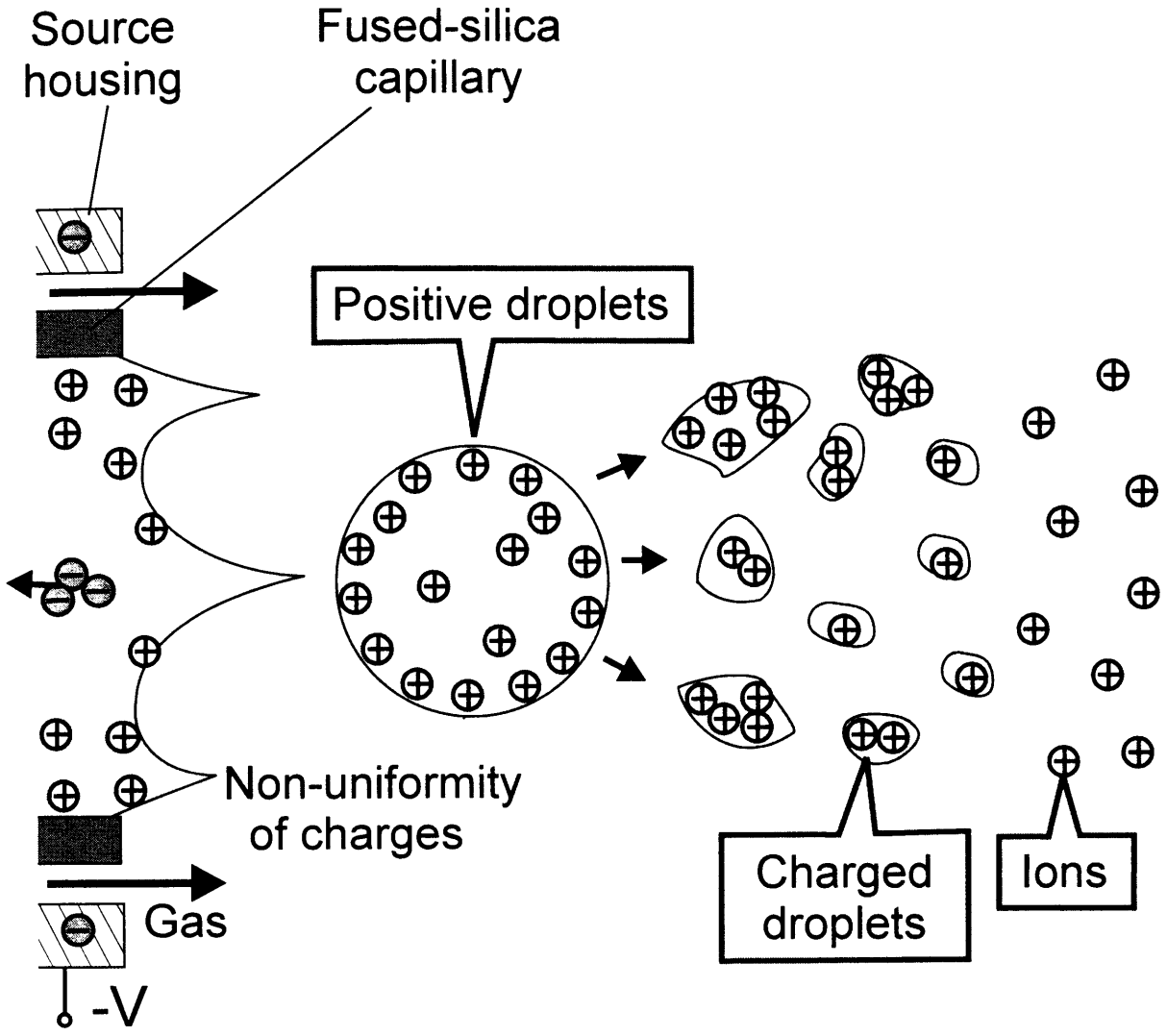

Figure 2.5 Schematic diagram of ion formation in the sonic spray when an electric field is applied to the solution. The non-uniformity of the charge density arises in the solution when an electric field is applied to the solution, and the concentration of ions with a polarity opposite to that of the applied electric field is increased at the solution surface. Therefore, the charge density of the produced droplets is increased, so that multiply-charged ions are produced.

The charge of the sprayed gas can be measured as an ion current by detecting the sprayed gas with an electrode. Figure 2.6 shows the dependence of the ion current on the voltage (V) to be applied to the source housing set around the fused-silica capillary. All charged species in the spray were detected with a 155-mm-long stainless steel tube with an inner diameter of 35 mm, 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. This ion current was obtained from a 5% acetic acid solution (water/methanol/acetic acid = 47.5/47.5/5%, v/v/v) under the condition of a sonic gas flow. The ion current at V = 0 was zero, and an ion current with polarity opposite to that of the voltage was detected. It was almost fully saturated above 1.2 kV or below -1.2 kV. At such voltages, the concentrations of ions with the same polarity are likely to be saturated at the solution surface. Therefore, high-charge-density droplets with a charge opposite to the voltage were produced by applying the voltages above 1.2 kV or below -1.2 kV. Note that although the sprayed gas might not be charged at V = 0, almost the same amounts of positively and negatively charged droplets were formed.

Thus, since the charge density of the droplets can be increased, the ionization efficiency from the charged droplets is increased. Also, multiply-charged ions with over three charges are produced by this method. Typical mass spectra of multiply-charged ions of proteins are shown in Figs. 2.7 and 2.8. These were obtained from 1-μM solutions of cytochrome-C (Fig. 2.7) and insulin (Fig. 2.8) containing 5% acetic acid (water/methanol/acetic acid = 47.5/47.5/5%, v/v/v) by applying a voltage of -1.2 kV at a solution-flow rate of 30-μL/min and a gas flow rate of 3 L/min. In Fig. 2.7, multiply-charged ions whose charge distribution ranged from 13+ to 19+ were observed. In Fig. 2.8, multiply-charged ions with 5+ and 6+ charges were also observed. Using this method,

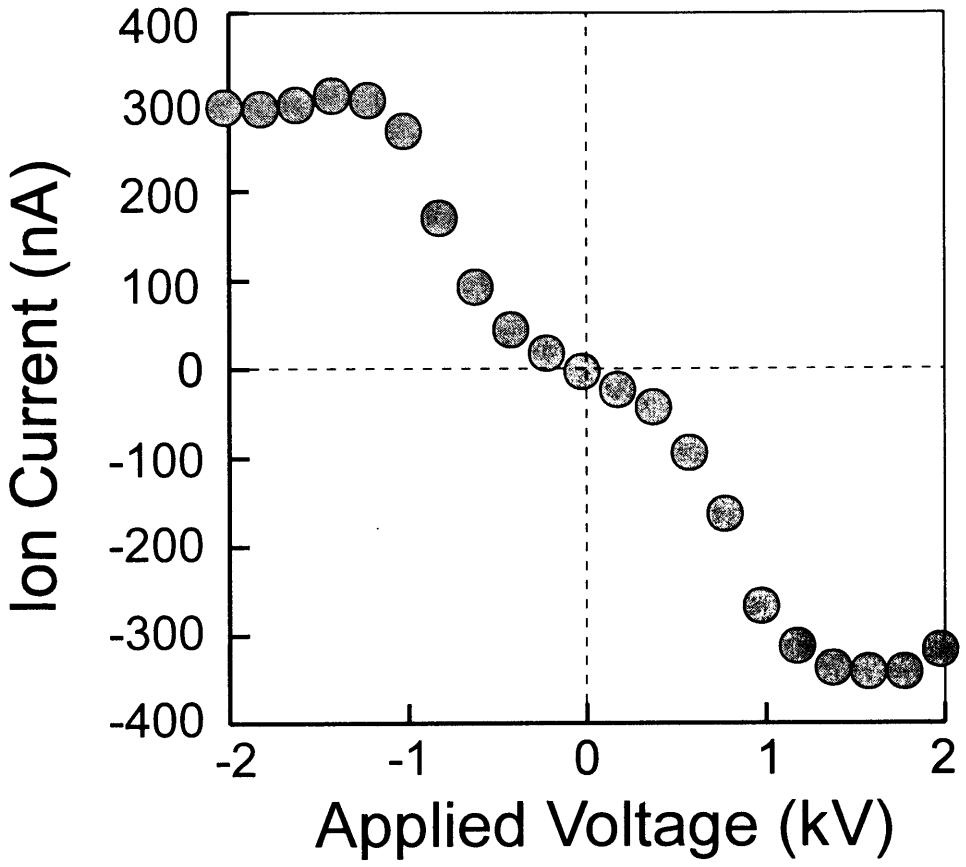

Figure 2.6 Ion current as a function of the voltage applied to the source housing. The result was obtained from a 47.5/47.5/5.0 methanol/water/acetic acid solution. The solution- and gas-flow rates were 30 μL/min and 3 L/min, respectively.

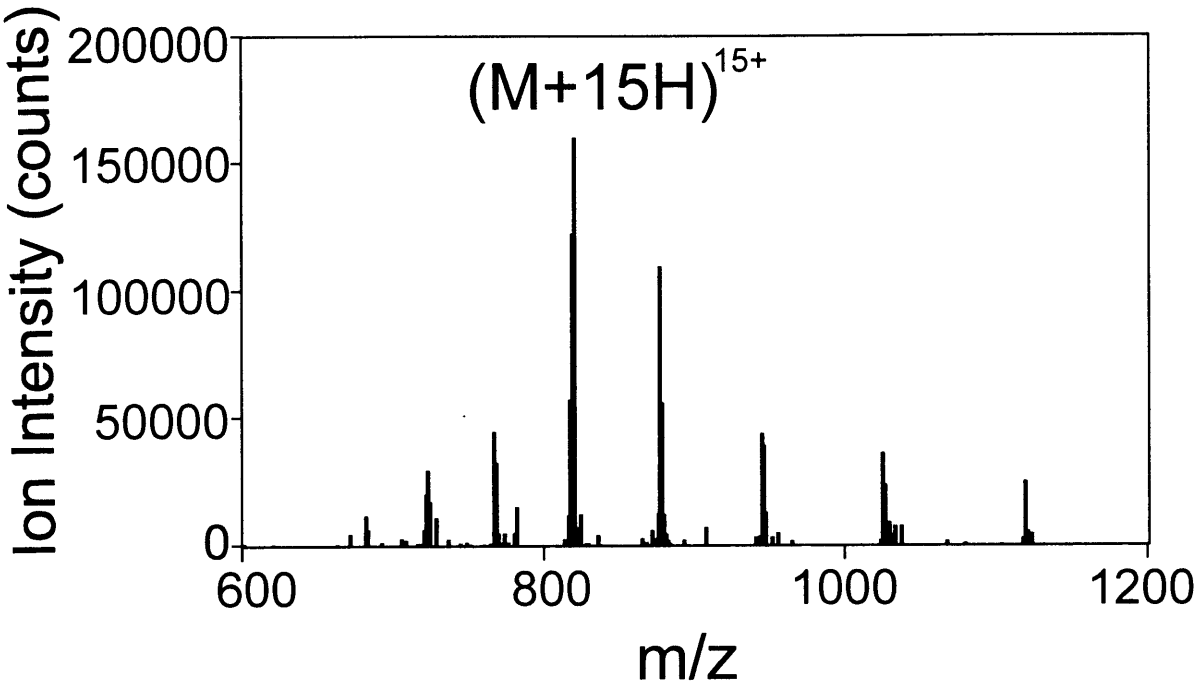

Figure 2.7 Mass spectrum from a 47.5/47.5/5 methanol/water/acetic acid solution of cytochrome-c at a solution flow rate of 30 μL/min. The solution concentration was 1 μM.

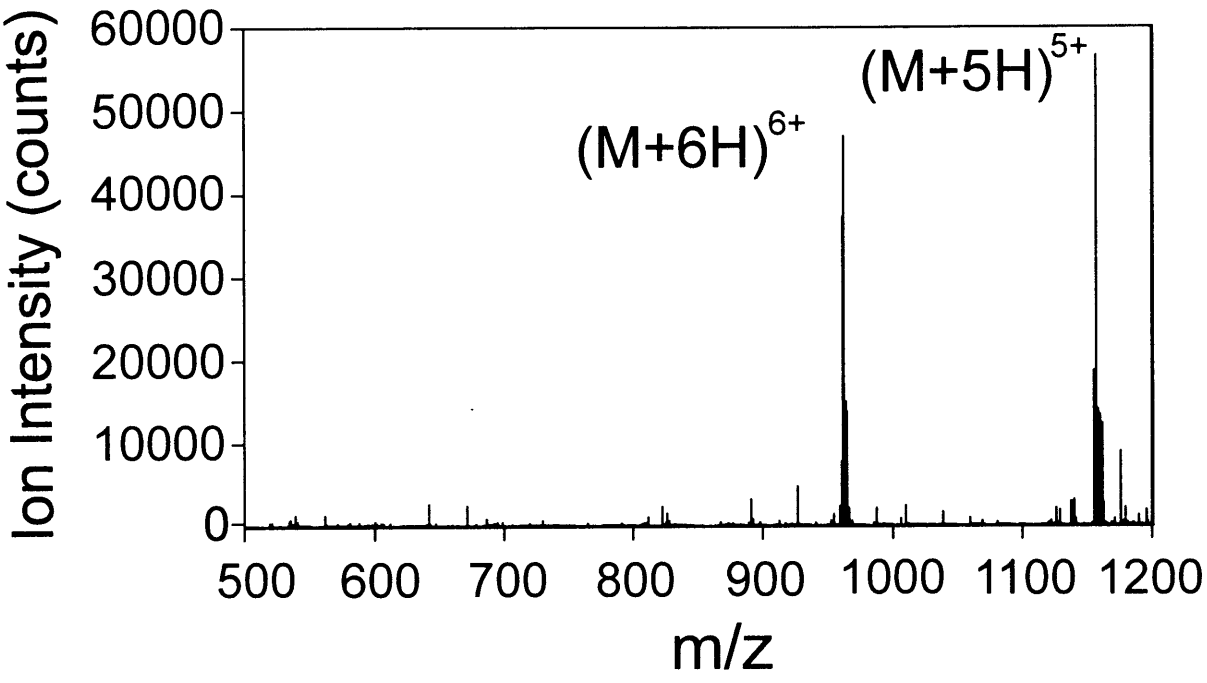

Figure 2.8 Mass spectrum from a 47.5/47.5/5 methanol/water/acetic acid solution of insulin at a solution flow rate of 30 μL/min. The solution concentration was 1 μM.

large molecule such as proteins, whose molecular weight are above 10,000, can be analyzed using SSI as with ESI and IS.

2.3 Research Strategy for Liquid Separation Techniques/ Mass Spectrometry Using Sonic Spray Ionization

The requirements for an ideal interface for LC/MS and CE/MS are as follows:

(1) it must be capable of analyzing a wide range of analyzable compounds, whether volatile, nonvolatile, or thermolabile compounds, and large molecules, such as proteins, can be analyzed; furthermore, it must be able to produce both positive and negative ions.

(2) a wide range of solution-flow rates is needed; an interface for LC/MS must be used at solution-flow rates ranging from 10 to 1000 μL/min, and an interface for CE/MS must be used at under 0.1 μL/min.

(3) there must be no limitation on the choice of buffer solution; nonvolatile buffers, such as phosphate, which are widely used as a mobile-phase buffer for CE, should be usable.

Although ESI can be used for the analysis of proteins since it can produce multiply-charged ions, it cannot be used at high solution-flow rates. APCI, which can be used at high solution-flow rates, cannot be used for protein analysis. Though IS can also be used at high solution-flow rates, multiply-charged ions cannot be produced at such solution-flow

rates. Therefore, neither interface technique satisfies both requirements (1) and (2). Also, LC/MS is often used to analyze the pesticide content in local bodies of water or drinking water. However, it is difficult to simultaneously analyze all pesticides whose concentrations in drinking water are regulated, because some thermolabile pesticides cannot be analyzed by the same technique as used for thermostable pesticides. Furthermore, the use of nonvolatile buffers, such as phosphate, which are generally used in CE, is generally avoided in CE/MS.

SSI, as described above, was an attractive potential interface for LC/MS and CE/MS, since it satisfied requirements (1) and (3), although the negative-ion formation had not yet been confirmed. However, since the solution flow rates in SSI ranged from 10 to 100 μL/min, it could not be directly coupled with LC or CE. One way to enable coupling was to control the solution-flow rates by using a splitter or a sheath liquid which would supply compensating liquid. However, such a configuration would be more difficult to operate, and the ion intensity of sample ions would be decreased because sample solutions would be lost or diluted by the splitter or sheath liquid.

Therefore, the purposes of this research was to confirm negative-ion formation in SSI and expand the range of solution-flow rates to enable coupling the SSI interface with a conventional LC, a semi-micro LC, or CE directly without use of a splitter or sheath liquid. The negative-ion formation is described in chapter 3. Direct coupling to semi-micro LC and MS, the improvement of SSI to enable high solution-flow rates with which conventional LC can be performed, and the coupling of CE and MS by using SSI are described in chapters 4, 5, and 6, respectively. The author has stated the goal whose achievement is described in each chapter: the goal in chapter 4 is to demonstrate the practicability of SSI by analyzing a mixture containing themolabile and themostable

pesticides simultaneously by semi-micro LC/MS, the goal in chapter 5 is to produce multiply-charged ions of a protein at a high solution-flow rate above 1 mL/min, and the goal in chapter 6 is to demonstrate analysis using a high-concentration phosphate buffer (above 10 mM) as the mobile-phase in CE/MS.

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3. Negative-ion Formation in Sonic Spray Ionization

The author has confirmed that negative-ion formation occurs in sonic spray ionization (SSI) and has identified a solution composition that increases the efficiency of negative-ion formation. The production of negatively charged droplets in SSI had already been confirmed by measuring the ion current of the sprayed gas. Furthermore, the negatively charged sprayed gas in SSI was stable and there was no risk of discharge which would interfere with ion formation as sometimes happens with the electrospray ionization (ESI) and ion spray (IS) techniques. However, the formation of negative ions had not been confirmed by mass spectrometry. In this research, the author also found that the efficiency of negative-ion formation in SSI was increased by adding three percent of ammonia to the sample solution. With this sample solution, the negative ions of nucleotides were analyzed by SSI.

3.1 Introduction

Liquid chromatography / mass spectrometry (LC/MS) and capillary electrophoresis / mass spectrometry (CE/MS) are powerful tools for analyzing mixtures, e.g. pesticides, proteins, or neurotransmitters, in various fields of science. To combine a liquid-phase separation tool such as an LC or CE with a mass spectrometer, the spray ionization technique used to provide an interface between the liquid-phase separation tool and the mass spectrometer is an important consideration. Spray ionization techniques such as atmospheric pressure chemical ionization (APCI) [1], electrospray ionization (ESI) [2, 3], ion spray (IS) [4], and atmospheric pressure spray (APS) [5] have been developed for LC/MS and CE/MS. Since some biological compounds, e.g. nucleotides, DNA, and neurotransmitters, are negatively charged, the analysis of negative ions is important, and of these techniques, only APCI, ESI, and IS can be used to produce negative ions. However, thermolabile compounds such as biological compounds cannot be analyzed by APCI, since the solution is nebulized by heat. These compounds can be analyzed by ESI or IS, but a high negative voltage must then be applied to the metallic capillary of the interface in which the sample solution flows to produce negative ions, and a discharge may occur due to electron emission from the tip of the capillary [6]. Such a discharge may interfere with by the ion formation.

In SSI, both volatile and nonvolatile compounds can be ionized. Since heat is not needed to produce ions, thermolabile compounds are especially readily analyzed. Furthermore, to apply an electric field to the solution in the sample-introduction capillary by applying a voltage to the source housing set around the capillary, the charge density of

the charged droplets can be increased, so that multiply-charged ions with over three charges were produced [9, section 2.2].

In SSI, a voltage of 1-2 kV, which is lower than that needed for ion formation in ESI and IS, is applied to the source housing set around the capillary. The production of negatively charged droplets, but not of negative ions, had already been confirmed by measuring the ion current of the sprayed gas [9, section 2.2]. Therefore, the author confirmed the production of negative ions with a quadrupole mass spectrometer, and changed the composition of the sample solution to increase the ionization efficiency of negative ions.

3.2 Experimental Section

Figure 3.1 is a cross-sectional view of the sonic spray interface. A fused-silica sample-introduction capillary (0.1-mm i.d., 0.2-mm o.d, GL Science, Tokyo) was fixed in a stainless-steel capillary (0.25-mm i.d., 1.7-mm o.d.) to enable it to be accurately positioned in the duralumin source housing, since the fused-silica capillary was flexible. A solution was pumped into the capillary at a flow rate of 30 μL/min. The end of the capillary extended 0.2 mm beyond a duralumin orifice (0.4-mm i.d.), and the center axes of the fused-silica capillary and the orifice were aligned. Nitrogen gas was used to pressurize the source housing, causing a gas flow from the orifice to the atmosphere. The flow rate of the

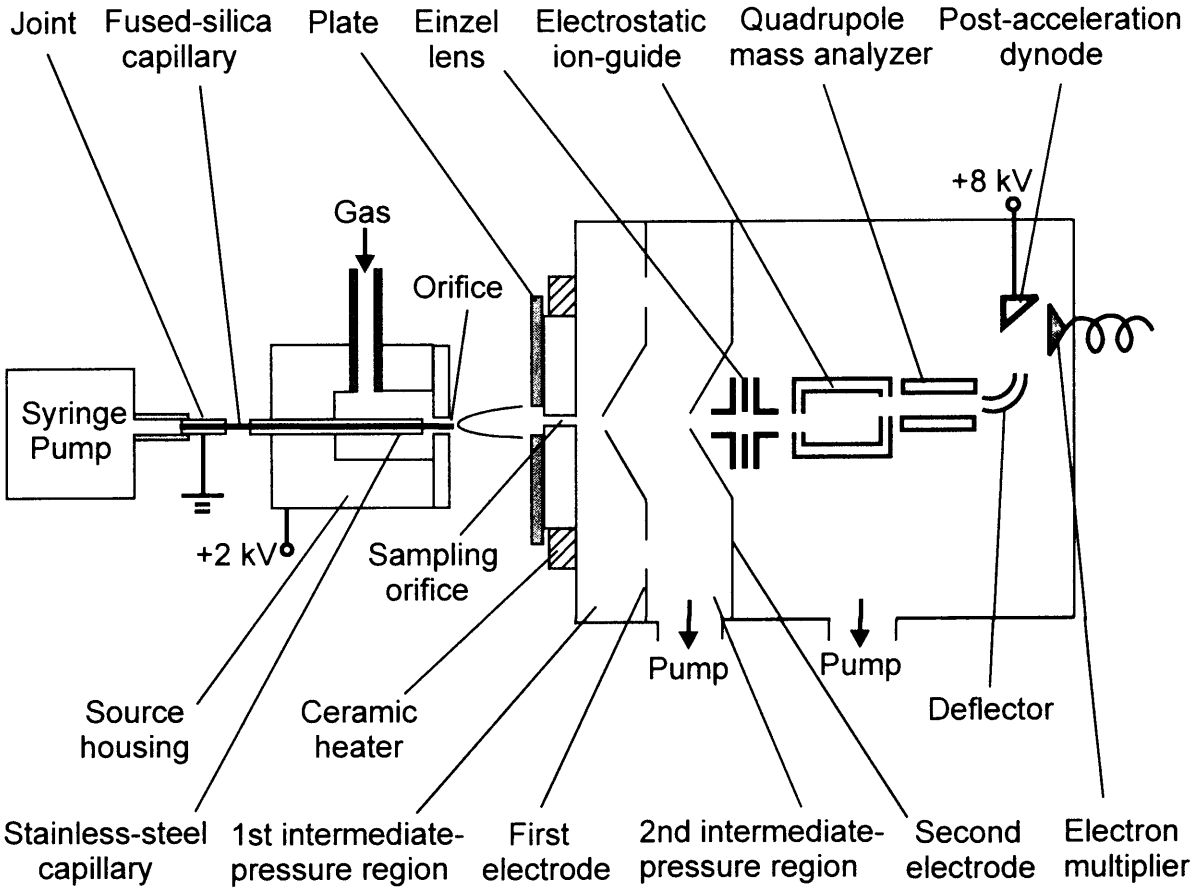

Figure 3.1 Cross-sectional view of the sonic spray interface.

nitrogen gas in the standard state, as determined with a mass-flow controller (5850E, Brooks Instrument, Tokyo), was 3 L/min. A spray was thereby generated in which droplets and free ions were produced. A voltage of 2 kV was applied to the orifice and the source housing, which were isolated from the solution by the capillary, to increase the charge density of droplets produced by the gas flow [9, section 2.2]. The electrical potential of the solution in the capillary was held at ground potential through a stainless steel joint. The sampling orifice (0.25-mm i.d., 25 mm long) of the mass spectrometer was heated with a ceramic heater (30 W) to about 120°C and covered with a stainless steel plate with a 2-mm aperture to avoid cooling of the sampling orifice due to gas flow and droplet evaporation. The distance between the fused-silica capillary tip of the interface and the stainless steel plate was about 4.5 mm.

Ions produced at atmospheric pressure passed into the first intermediate-pressure region through the sampling orifice. The ions then passed into the second intermediate-pressure region through a 0.5-mm aperture in the first electrode. Finally, the ions passed into the mass analyzing region through a 0.2-mm aperture in the second electrode. (The details of the mass spectrometer have been described elsewhere [10].) An electrostatic ion guide [11] was set in front of a quadrupole mass analyzer in the mass analyzing region. The original setup was optimized for positive ion analysis, so the same absolute voltages with polarity opposite to the original setup were applied to the first and second electrodes, the einzel lens, the electrostatic ion guide, the quadrupole mass analyzer, the deflector, and the post-acceleration dynode. A drift voltage of -62 V was applied between the sampling orifice and the second electrode, and voltages of 8 and 2 kV were applied to the post-acceleration dynode and the electron multiplier, respectively.

3.3 Results and Discussion

Solution Conditions for Negative Ion Formation

A mass spectrum obtained from a glutamic-acid solution is shown in Fig. 3.2(a). The glutamic-acid solution was a 50:50 methanol/water solution, which was the same solution used for the previous singly and doubly charged positive-ion analysis, at a concentration of 100 μM. The deprotonated glutamic acid molecules were detected at m/z = 146. Generally, negative ions are unstable and their lifetime is shorter than that of positive ions because electrons associate with molecules due to the weak electron affinity of the molecules. Even so, this ion intensity was too low. Therefore, the author tried to increase the ionization efficiency of the glutamic acid by adding a volatile electrolyte to the sample solution; a volatile compound enhances solvent evaporation from the charged droplets, because the volatile compound desorbs from the droplets together with solvent molecules. Acetic acid was added to the sample solution at first because this increased the ionization efficiency of positive ions. However, although cluster ions of acetic acid molecules were strongly detected, the ion intensity of the analyte did not increase (Fig. 3.2(b)). Since acetic acid molecules are negatively charged, they would have taken charges away from the sample ions. Thus, adding volatile compounds that would not be detected as negative ions to the solution would increase the ionization efficiency of negative ions. Therefore, the author added ammonia to the glutamic-acid solution.

Figure 3.3 shows the dependence of the ion intensity of the glutamic acid on the concentration of ammonia added to the sample solution. The concentration of glutamic acid was 100 μM. The ion intensity reached a maximum at 3% of ammonia. This result

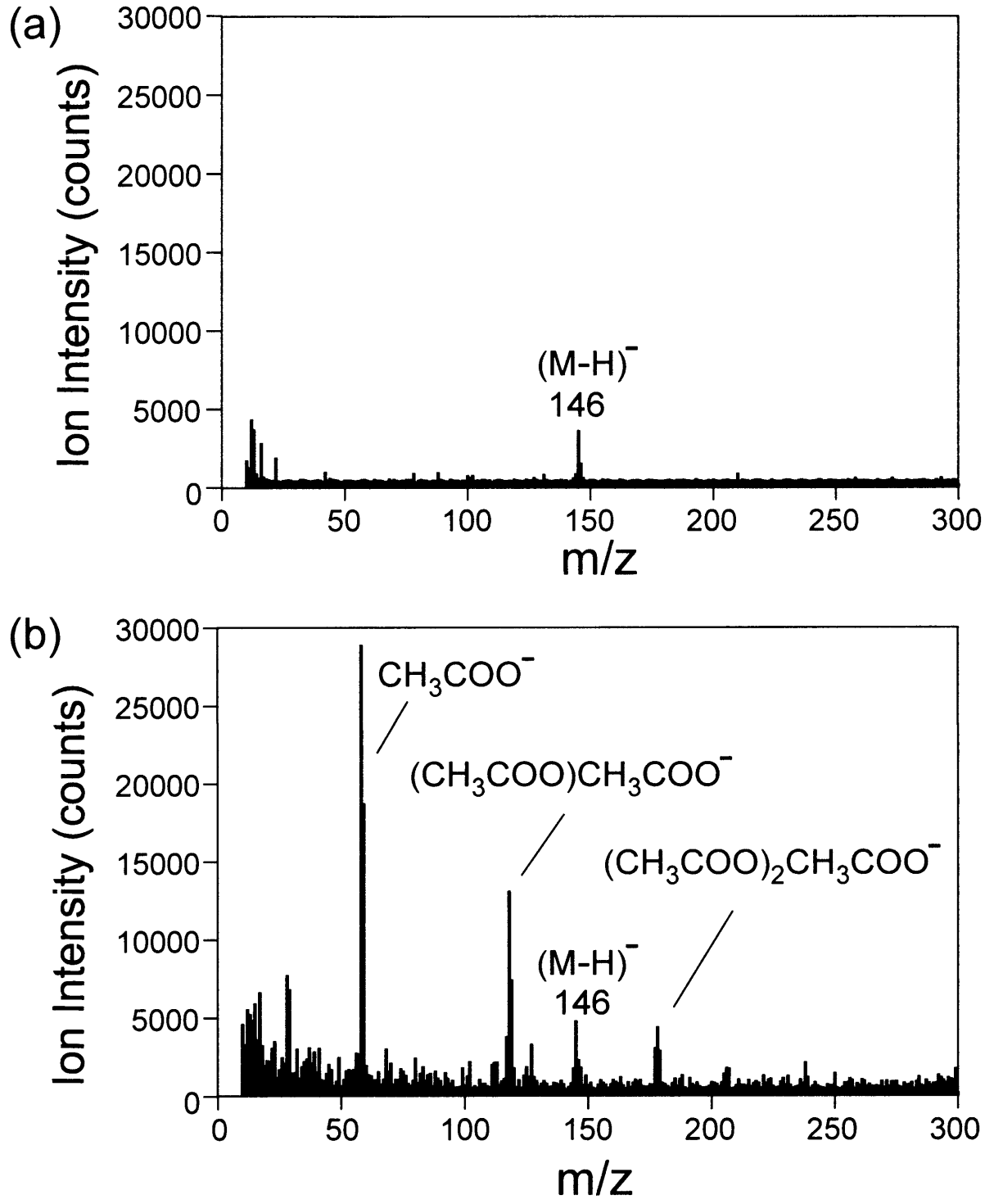

Figure 3.2 Mass spectra of glutamic acid obtained from (a) a 50:50 methanol/water solution and (b) a 47.5/47.5/5 methanol/water/acetic acid solution at a solution flow rate of 30 μL/min. The solution concentration was 100 μM.

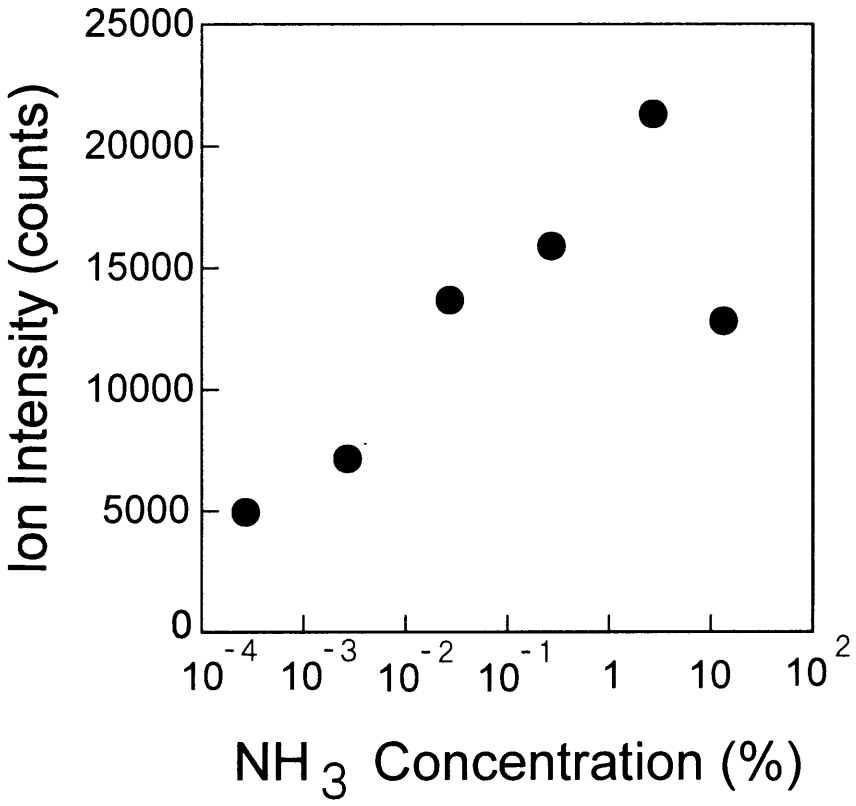

Figure 3.3 Dependence of the ion intensity of the glutamic-acid ion (m/z = 146) on the concentration of ammonia added to the solutions. The glutamic-acid concentration was 100 μM.

was explained as follows.

In SSI, when an electric field is applied to the solution in the capillary by applying a voltage to the source housing and the orifice, species with a charge opposite to the applied electric field gather at the solution surface. Thus, since the charge density opposite to the electric field increases at the solution surface, the charge density of droplets with a charge opposite to the electric field increases [9 and chapter 2.2]. In the same way, ions of a electrolyte added to the solution would also gather at the solution surface due to the electric field, so the charge density at the solution surface would be further increased by adding the electrolyte. Furthermore, when a volatile electrolyte such as acetic acid evaporates together with the solvent, charges are left in the charged droplets. This increases the charge density of the charged droplets and the ionization efficiency of the analyte. For positive-ion formation, adding 5% of acetic acid to the solution provided the greatest empirical increase in the ionization efficiency since the concentration of charges would be saturated at the surface of the solution and droplets at this concentration. Also, the concentration of charges would be reflected in the conductivity of the solution. Therefore, the author measured the solution conductivity of various solutions. Table 3.1 shows the conductivity of ammonia, acetic-acid, and sodium-hydroxide solutions. As shown in table 3.1, the conductivity of a 5% acetic-acid solution was about 2.5 mS/m, almost the same as the conductivity of a 3% ammonia solution, which was the most effective for increasing the ion intensity of glutamic acid. Therefore, solutions whose conductivity is about 2.5 mS/m appear to be the most suitable for ionization in SSI. However, though the conductivity of the 3-mM sodium-hydroxide solution was also about 2.5 mS/m, the ion intensity was low (data is not shown). Because sodium hydroxide is nonvolatile, it cannot enhance the evaporation of solvent from the droplets.

Table 3.1

Ammonia

Concentration (%)	0	0.0003	0.003	0.03	0.3	3	15
Conductivity (mS/m)	0.01	0.02	0.06	0.26	0.85	2.63	2.11

Acetic Acid

Concentration (%)	0	0.01	0.1	1	5	20
Conductivity (mS/m)	0.06	0.10	0.22	1.14	2.34	3.22

Sodium Hydroxide

Concentration (mM)	0	0.1	0.3	1	1.5	3	10
Conductivity (mS/m)	0.07	0.07	0.16	0.73	1.20	2.78	9.00

A typical mass spectrum obtained from a 100-μM glutamic-acid solution (water/methanol/ammonia = 49/50/1%, v/v/v) is shown in Fig. 3.4. Here, the effect of 1% of ammonia was almost the same as that of 3% of ammonia. The negative ion of glutamic acid was clearly detected at m/z = 146.

Mass Spectra of Nucleotides

Using the 1% ammonia solution described above, the author then analyzed nucleotides. Figure 3.5 shows mass spectra of adenosin-5'-mono-phosphate (AMP). The spectra of Figs. 3.5(a) and (b) were obtained from AMP, free acid and AMP, disodiumsalt, respectively. The solutions were 49:50:1 water/methanol/ammonia at a concentration of 100 μM. In Fig. 3.5(a), a singly charged AMP ion ($(M-H)^-$), a dimer of the AMP ion ($M(M-H)^-$), and a trimer of the AMP ion ($MM(M-H)^-$) were observed at m/z = 346, 693, and 1040, respectively. In Fig 3.5(b), though a singly charged AMP ion ($(M-H)^-$) and a sodiated AMP ion ($(M-2H+Na)^-$) were observed at m/z = 346 and 378, respectively, the dimer and trimer of the AMP ion were not observed.

Figure 3.6 shows mass spectra of adenosin-5'-diphosphate (ADP) and adenosin-5'-triphosphate (ATP). These were obtained from disodiumsalt chemicals. The solutions were 49:50:1 water/methanol/ammonia at a concentration of 100 μM. Singly and doubly charged ADP ions ($(M-H)^-$ and $(M-2H)^{2-}$) were observed at m/z = 426 and 215, respectively. Also, singly and doubly charged sodiated ions of ADP ($(M-2H+Na)^-$ and

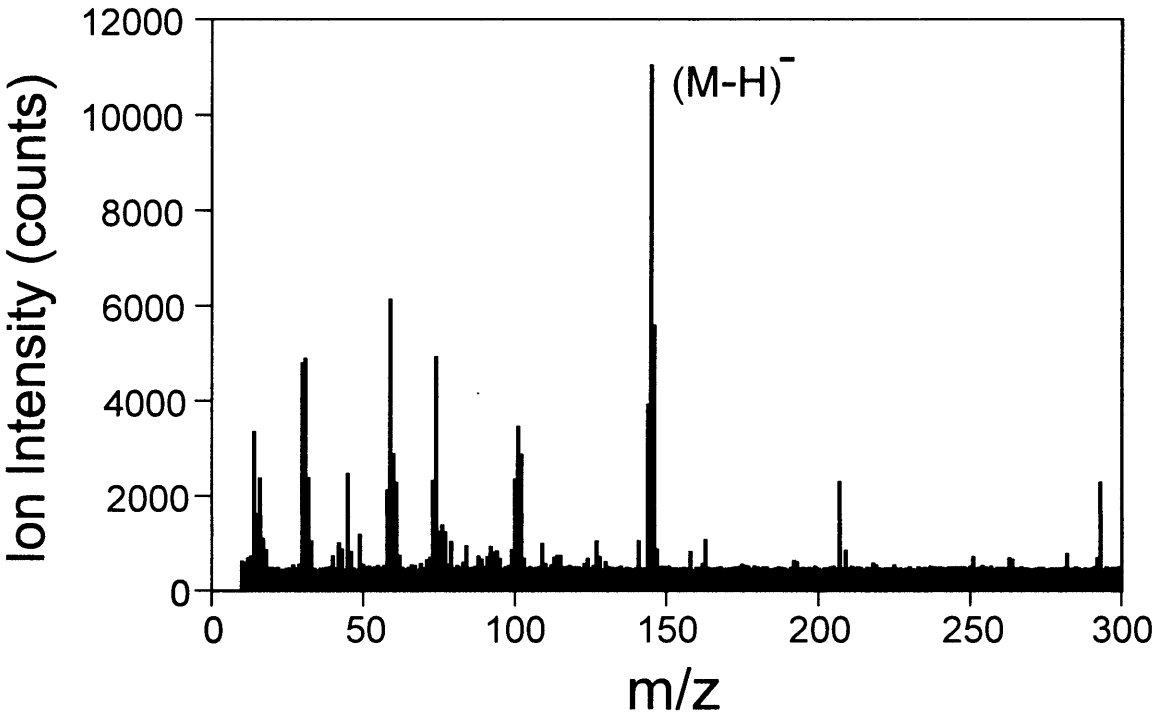

Figure 3.4 Mass spectrum of a glutamic acid obtained from a water/methanol/ammonia solution (49/50/1%, v/v/v) at a solution flow rate of 30 μL/min. The solution concentration was 100 μM.

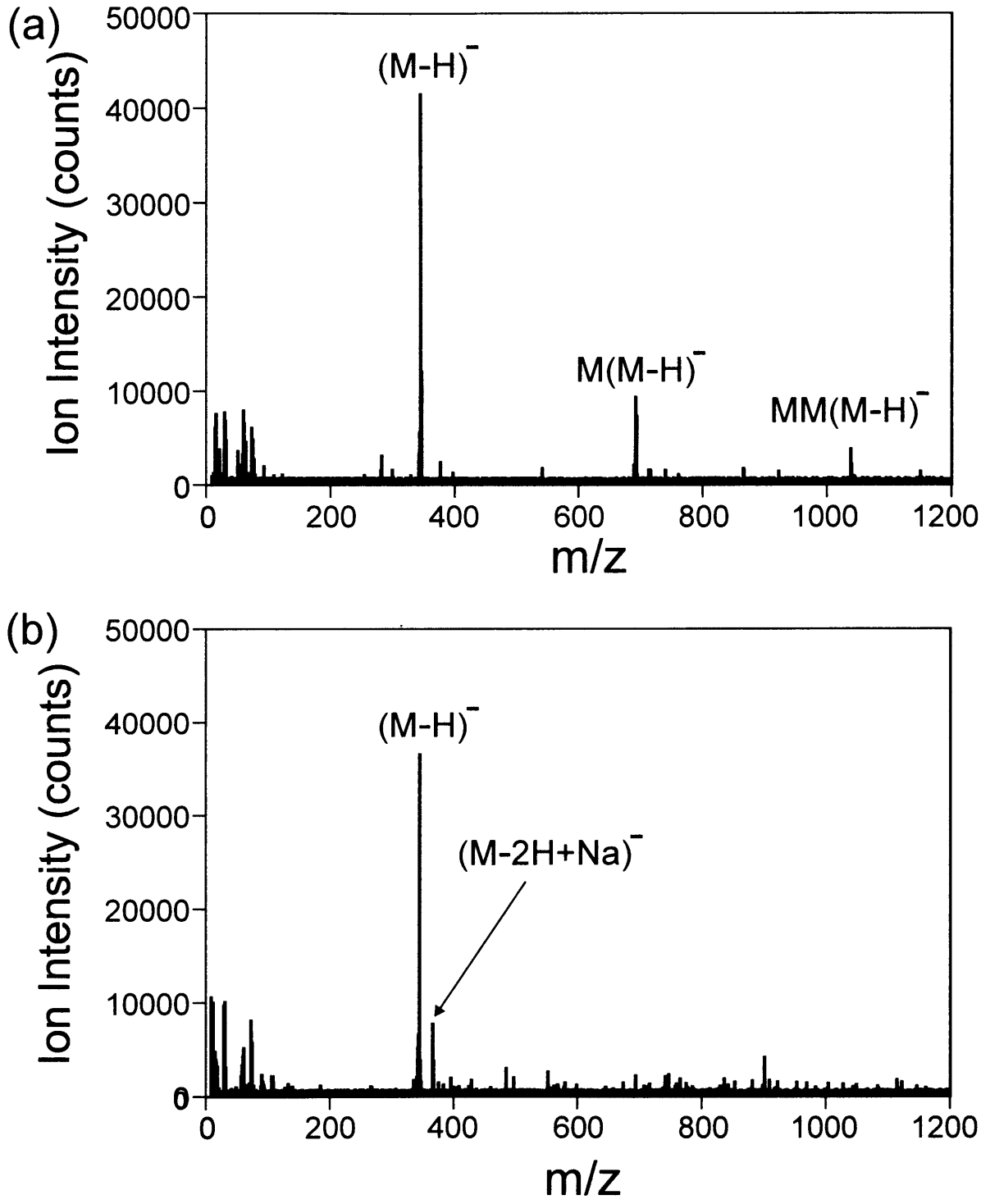

Figure 3.5 Mass spectra of (a) AMP free acid, and (b) AMP disodiate, obtained from water/methanol/ammonia solutions (49/50/1%, v/v/v) at a solution flow rate of 30 μL/min. The solution concentrations were 100 μM.

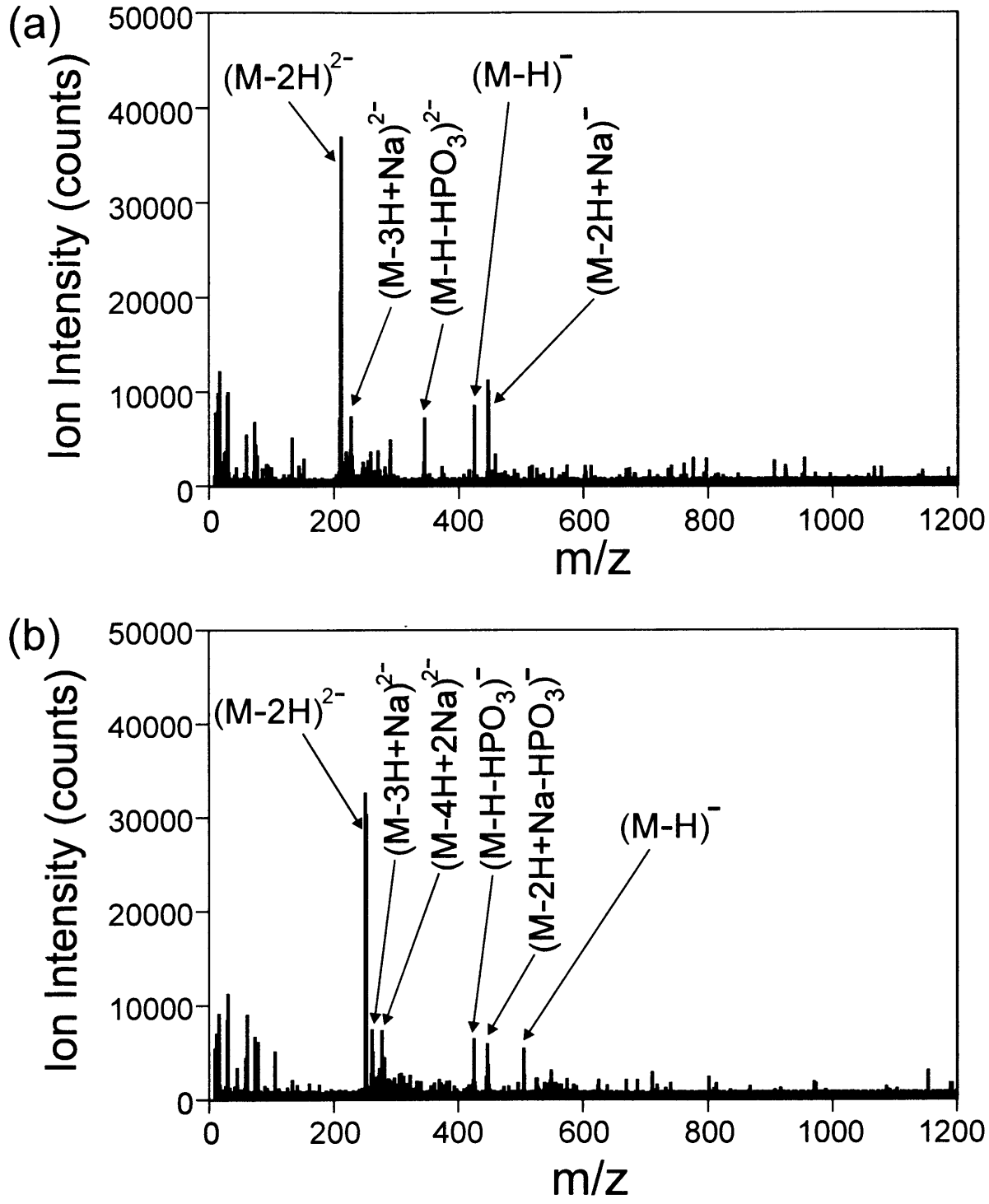

Figure 3.6 Mass spectra of (a) ADP disodiate, and (b) ATP disodiate, obtained from water/methanol/ammonia solutions (49/50/1%, v/v/v) at a solution flow rate of 30 μL/min. The solution concentrations were 100 μM.

$(M-3H+Na)^{2-}$) and the AMP ion, which would be contaminated in the standard sample, were detected at m/z = 447, 224, and 346, respectively. In Fig. 3.6(b), singly and doubly charged ATP ions ($(M-H)^-$ and $(M-2H)^{2-}$) were observed at m/z = 506 and 253, respectively. Doubly charged sodiated ions of ATP ($(M-3H+Na)^{2-}$ and $(M-4H+2Na)^{2-}$), the ADP ion, and the sodiated ADP ion, which would also be contaminated in the standard sample, were detected at m/z = 264, 280, 426, and 448, respectively.

With this solution, SSI can clearly be used for negative ion analysis. Furthermore, there is no discharge in SSI because the voltage needed for ionization is lower than that in ESI and IS. Therefore, SSI allows us to analyze a wide range of analyzable compounds that contain negatively charged species.

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4. Direct Coupling of a Semi-micro Liquid Chromatograph and a Mass Spectrometer Using Sonic Spray Ionization

Direct coupling of semi-micro liquid chromatography and sonic spray ionization mass spectrometry is demonstrated and shown to be a very powerful technique for pesticide analysis. Direct coupling was achieved by using a high flow rate of nitrogen gas in sonic spray ionization and a high operating temperature in the sampling orifice of the atmospheric-ionization mass spectrometer. The detection limit for simazine was about 300 fmol based on quantitative analysis in the range between 1 pmol and 1 nmol. A mixture of three pesticides was successfully analyzed using semi-micro liquid chromatography / mass spectrometry.

4.1 Introduction

The analysis of environmental-polluting compounds in solutions is becoming more important in analytical science. For example, the concentrations of pesticides in drinking water have recently come under stricter regulation in Japan. With the rapidly growing number of items that must be monitored, highly sensitive tools that can analyze mixtures are becoming more important. Liquid chromatography / mass spectrometry (LC/MS) has become one such tool for analyzing mixtures in a solution. The interface to combine the liquid-phase separation technique with mass spectrometry is very important, and the standard LC/MS interface is atmospheric pressure ionization (API) [1-7].

In the sonic spray ionization (SSI) interface for LC/MS [8,9], compounds are ionized by nebulizing sample solutions using only a high-speed gas flow at room temperature, but the solution flow rate (under 100 μL/min) was too low for semi-micro LC. One way to achieve compatibility would have been to use a splitter to control the rate of flow into the interface, but a better approach was direct coupling because it made the system easier to operate and enabled higher separation efficiency.

This chapter describes how the SSI operating conditions were modified to enable the direct coupling of semi-micro LC with SSI-MS, and examines the results from a pesticide-mixture analysis using semi-micro LC/SSI-MS.

4.2 Comparison of SSI and APCI in Pesticide Analysis

One of the merits of SSI is its high ionization efficiency. The author began by comparing SSI in its original configuration [8,9], where the solution-flow rate was 30 μL/min, to a common ionization technique, e.g. atmospheric pressure chemical ionization (APCI) [1], which is a form of API. A commercial APCI interface taken from a conventional LC/MS system (M-1000, Hitachi, Tokyo) was used at a typical solution-flow rate of 1000 μL/min. Simazine and thiobencarb, which are regulated pesticides in Japan, were used as samples for the comparison. However, thiuram, which is also regulated and a typical thermolabile pesticide, was not used as a sample because it is difficult to analyze by APCI. The resolution of SSI and APCI is compared in Figs. 4.1 and 4.2. Figure 4.1 shows the mass spectra obtained from the 1-μM simazine solution (methanol/water = 50/50%, v/v), and Fig. 4.2 shows the mass spectra obtained from the 1-μM thiobencarb solution (methanol/water = 50/50%, v/v). Protonated molecules of simazine and thiobencarb were detected at m/z = 202 and 258, respectively. Protonated water molecules, protonated methanol molecules, two types of water and methanol clusters, dimers of methanol, and trimers of methanol were also detected at m/z = 19, 33, 51 and 115, 65, and 97, respectively. Though the intensities of ions and clusters created from solvent molecules were almost the same as the intensities of the sample ions with SSI (Figs. 4.1(a) and 4.2(a)), these intensities were much higher than the intensities of the sample ions with APCI (Figs. 4.1(b) and 4.2(b)). Furthermore, the signal-to-noise ratio (S/N) of the simazine ion with SSI and with APCI was 335 and 96, respectively, and the S/N of the thiobencarb ion with SSI and with APCI was 579 and 180, respectively. These results clearly show that the

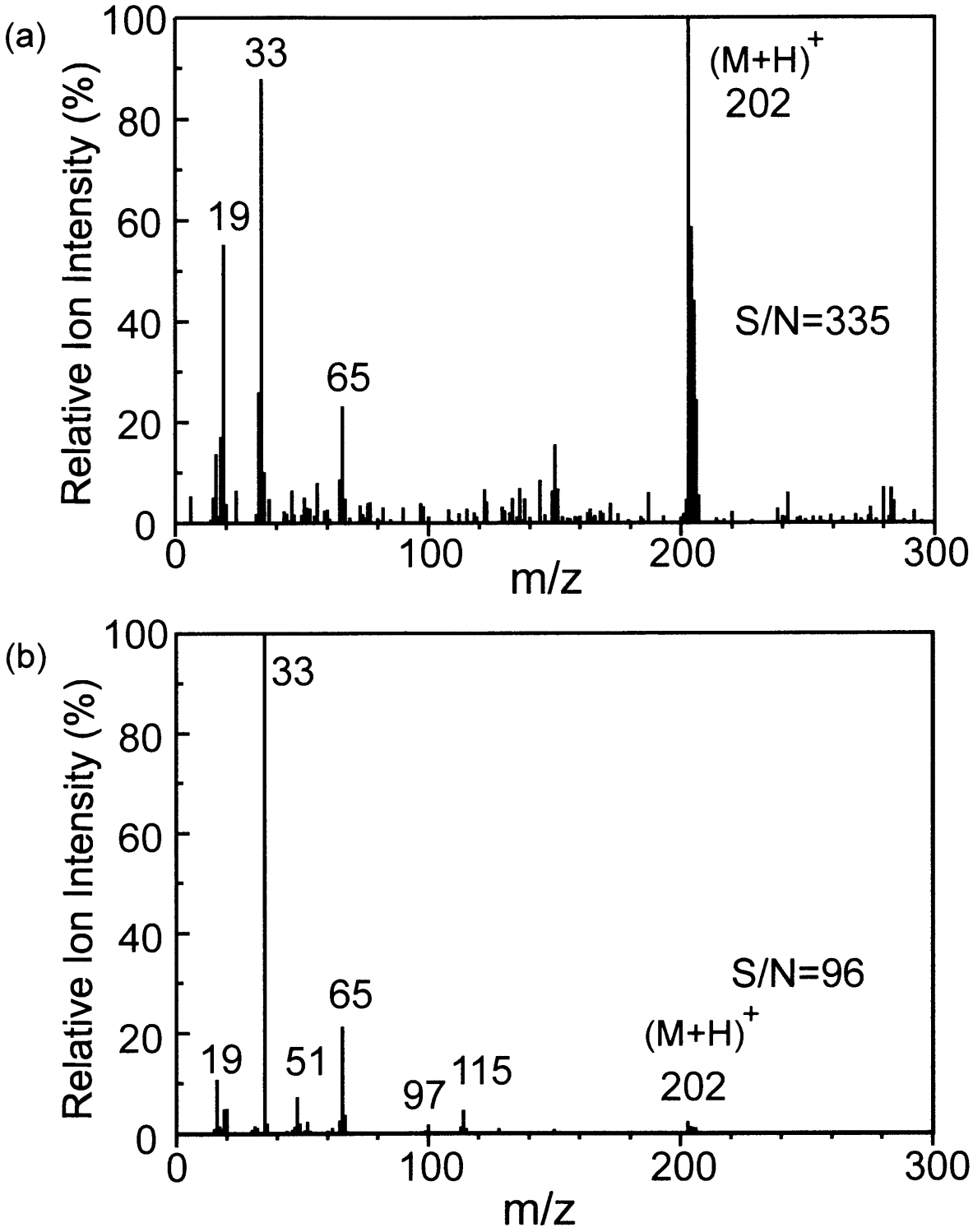

Figure 4.1 Mass spectra obtained from the simazine solution (methanol/water = 50/50%, v/v) by (a) SSI and (b) APCI at solution flow rates of (a) 30 μL/min and (b) 1000 μL/min. The solution concentration was 1 μM.

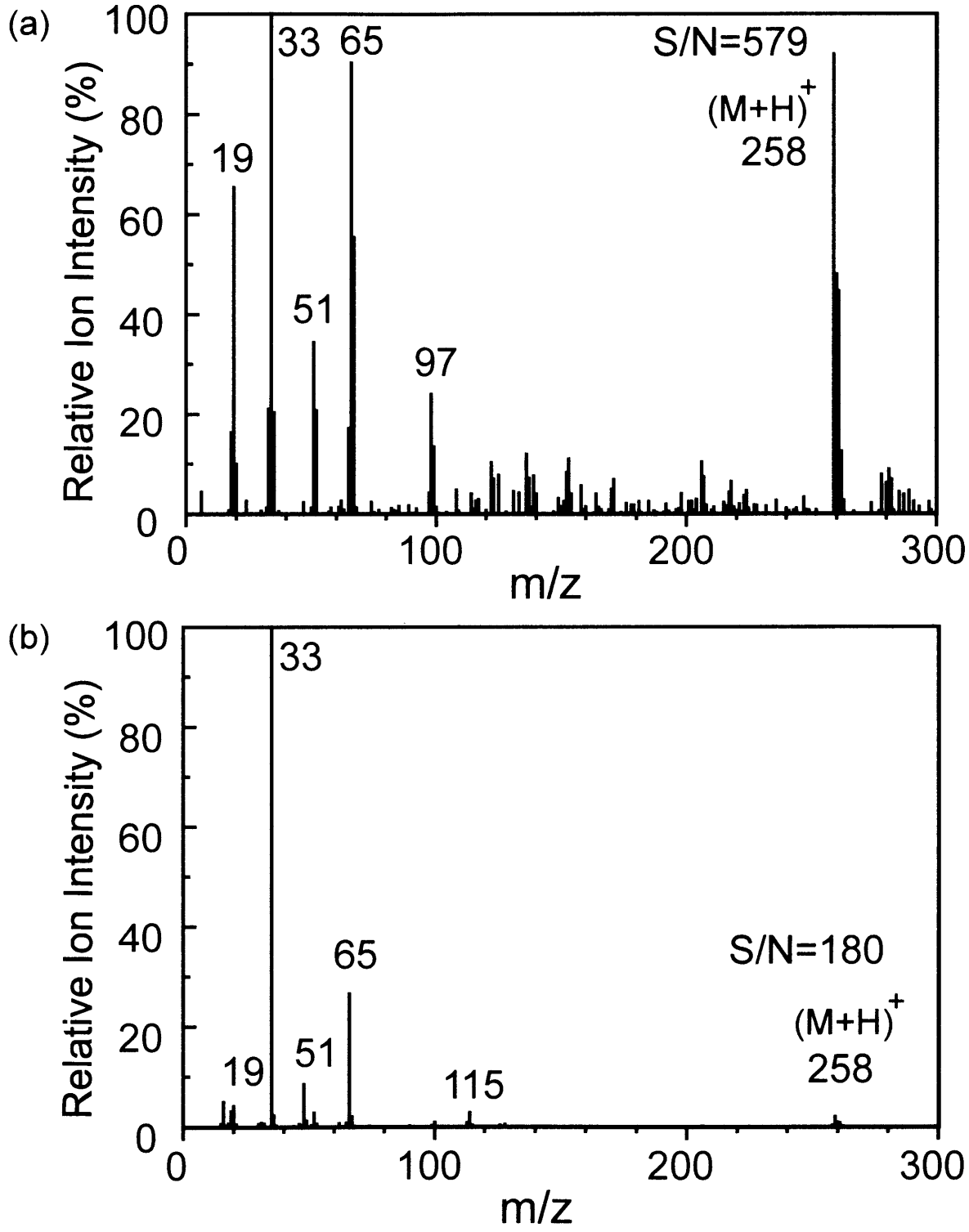

Figure 4.2 Mass spectra obtained from the thiobencarb solution (methanol/water = 50/50%, v/v) by (a) SSI and (b) APCI at solution flow rates of (a) 30 μL/min and (b) 1000 μL/min. The solution concentration was 1 μM.

ionization efficiency of SSI is much higher than that of APCI for these types of compounds.

4.3 Experimental Section

Figure 4.3 is a cross-sectional view of the sonic spray interface which is similar to the interface described in section 3.2 of the previous chapter. (The details have been described elsewhere[8,9].) For spectra measurements, a solution was pumped into the fused-silica capillary at a flow rate ranging from 100 to 200 μL/min. The potential of the source housing was set to the ground level. In this case, the flow rate of the nitrogen gas in the standard state was 6 L/min.

For the LC/MS measurements, the sample mixture was separated with a semi-micro LC column (TSK-GEL PTH PAK, TOSOH, Tokyo Japan). The mobile phase was pumped by a Hitachi L-6200 LC pump into the fused-silica capillary at a flow rate of 200 μL/min. The mobile phase was water/methanol (60/40%, v/v) for the first ten minutes, then a linear gradient from 60% water/40% methanol to 100% methanol over ten minutes was used for the separation.

The distance between the fused-silica capillary tip of the interface and the sampling orifice of the mass spectrometer was 3 mm. The sampling orifice of the mass spectrometer was heated with a 50-W ceramic heater to 120-130°C. As in the previous configuration,

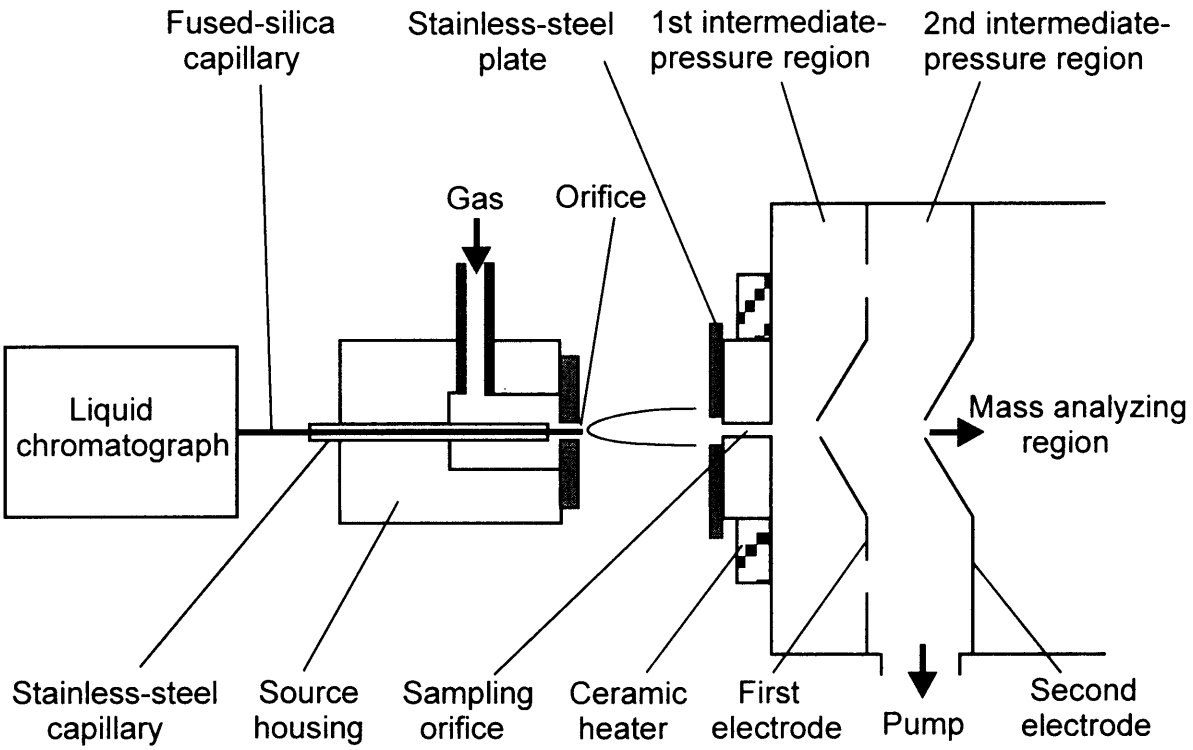

Figure 4.3 Cross-sectional view of the sonic spray interface.

ions produced at atmospheric pressure passed into the first intermediate-pressure region through the sampling orifice, then passed into the second intermediate-pressure region through a 0.5-mm aperture in the first electrode before passing into the mass analyzing region through a 0.2-mm aperture in the second electrode. In this case, though, a drift voltage of 10 V was applied between the sampling orifice and the first electrode, and another drift voltage of 30 V was applied between the first and second electrodes.

4.4 Results and Discussion

Under the initial operating conditions [9], intense analyte ions were obtained by the mass spectrometer at a solution-flow rate of around 30 μL/min; however, at solution-flow rates ranging from 100 to 200 μL/min, intense ions could not be obtained. Therefore direct coupling of semi-micro LC and SSI-MS could not be achieved. At a solution-flow rate of around 30 μL/min, charged droplets were produced by spraying a solution, and ions were produced from the charged droplets by evaporation of the charged droplets in the atmosphere. At a solution-flow rate of over 100 μL/min, ions as well as charged droplets were apparently produced in the sprayed gas when using a nitrogen-gas flow rate of 3 L/min, because the size of the charged droplets produced by the sonic gas flow (about 3 L/min) was very small, i.e. about 1 μm [10], and these droplets would quickly evaporate in the atmosphere. However, the density of the gas-phase solvent molecules in the sprayed

gas at 3 L/min was quite high, so ions were likely associated with solvent molecules and probably formed large clusters and charged droplets as they cooled due to the adiabatic expansion when they were introduced into the vacuum region through the sampling orifice of the mass spectrometer [11]. The tendency to associate was also enhanced by the cooling of the sampling orifice due to the evaporation of droplets from the solution being delivered at a high solution-flow rate. Therefore, the author investigated the operating conditions that affect the sampling orifice temperature and the nitrogen gas-flow rate that will enable direct coupling of semi-micro LC with SSI-MS.

The temperature needed in the 25-mm-long sampling orifice to prevent the association of ions and solvent molecules was empirically determined as about 120°C [8]. However, the sampling orifice is quickly cooled in SSI due to the evaporation of droplets from the solution and the high-speed gas flow. Thus, the author measured the temperature change in the sampling orifice at a 200-μL/min solution flow rate. As shown in Fig. 4.4, 40 minutes after the solution flow started, the temperature of the sampling orifice when heated with 30- and 50-W ceramic heaters was below 80°C and 120°C, respectively. Thus, the sampling orifice had to be heated with at least a 50-W ceramic heater to maintain the required temperature of 120°C for semi-micro LC.

Also, the increased gas flow rate used to spray the solution caused dilution and a decrease in the density of solvent molecule in the sprayed gas. Figure 4.5 shows the relationship between the ion intensity of a protonated simazine molecule (m/z = 202) and the gas flow rate at a solution-flow rate of 200 μL/min. In this experiment, the solution concentration was 1 μM (water/methanol = 50/50%, v/v) and the sampling orifice was heated with a 50-W ceramic heater. The ion intensity reached a maximum at a gas flow rate of about 5-6 L/min. In SSI, the ionization efficiency reaches a maximum at the sonic

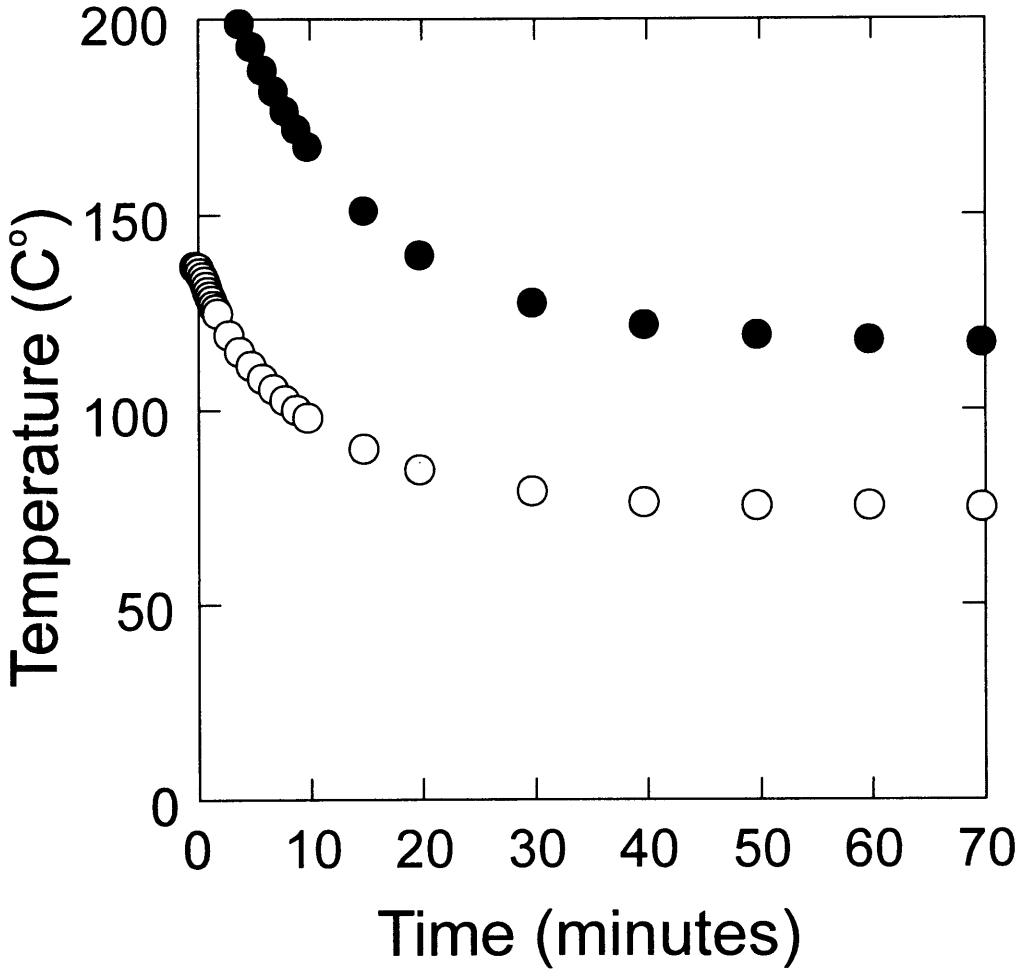

Figure 4.4 Temperature change in sampling orifice heated with 30- (O) and 50-W (●) ceramic heaters at a 200-μL/min solution flow rate and a 3-L/min gas flow rate.

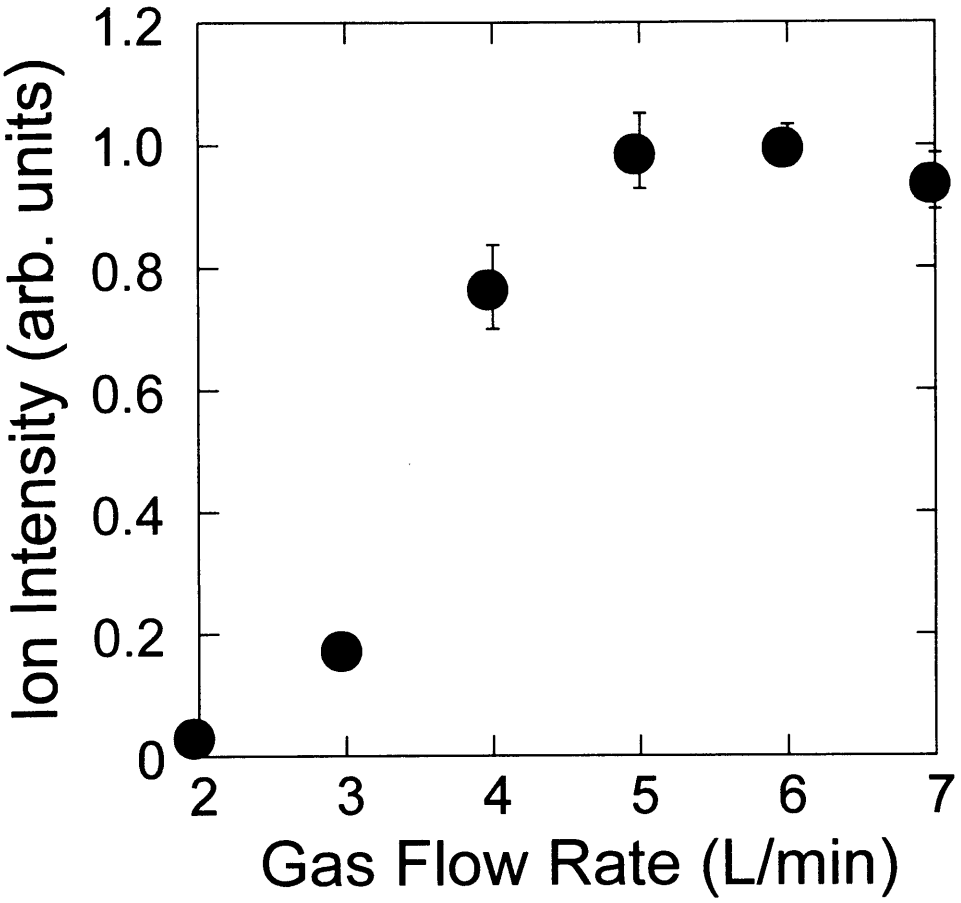

Figure 4.5 Relation between ion intensity of the protonated simazine molecule (m/z = 202) at a 200-μL/min solution-flow rate and various nitrogen gas flow rates. The simazine concentration was 1 μM.

gas velocity, i.e., a gas flow rate of 3 L/min. However, since the density of solvent molecules in the sprayed gas was too high at 3 L/min, the ion intensity decreased due to association with solvent molecules in the mass spectrometer. At a gas flow rate of 6 L/min, the density of solvent molecules in the sprayed gas was sufficiently decreased by the higher flow rate of the gas, though the ionization efficiency was not the highest. Therefore, a gas flow rate of about 6 L/min was suitable for performing experiments at the flow rate needed for semi-micro LC/SSI-MS.

Thiuram was used as a model compound because it is a typical thermolabile compound and is difficult to analyze by using gas chromatography (GC) or GC/MS. A typical mass spectrum of thiuram obtained by SSI-MS is shown in Fig. 4.6. The thiuram solution was 50:50 methanol/water at a concentration of 10 μM; the gas flow rate was 6 L/min and the solution flow rate was 100 μL/min. Protonated and sodiated molecules of thiuram were detected at m/z = 241 and 263, respectively. The random noise in the spectrum was caused by detecting charged droplets whose m/z values were too high to be affected by the electric field of the quadrupole mass spectrometer.

The dynamic range of quantitative analysis using SSI-MS was then analyzed under these new operating conditions. The calibration curve for simazine is shown in Fig. 4.7. The author repeatedly injected 10-μL sample solutions with different concentrations without a column, and the protonated molecules (m/z = 202) were detected by using the selected ion monitoring (SIM) mode. The flow rate of the mobile phase (water/methanol, 50%/50%) was set at 100 μL/min. Good linearity was obtained in the range between 1 pmol and 1 nmol. Based on this result, the estimated detection limit, with a S/N ratio of 2, for simazine was about 300 fmol. This SSI method was thus sensitive enough to detect simazine.

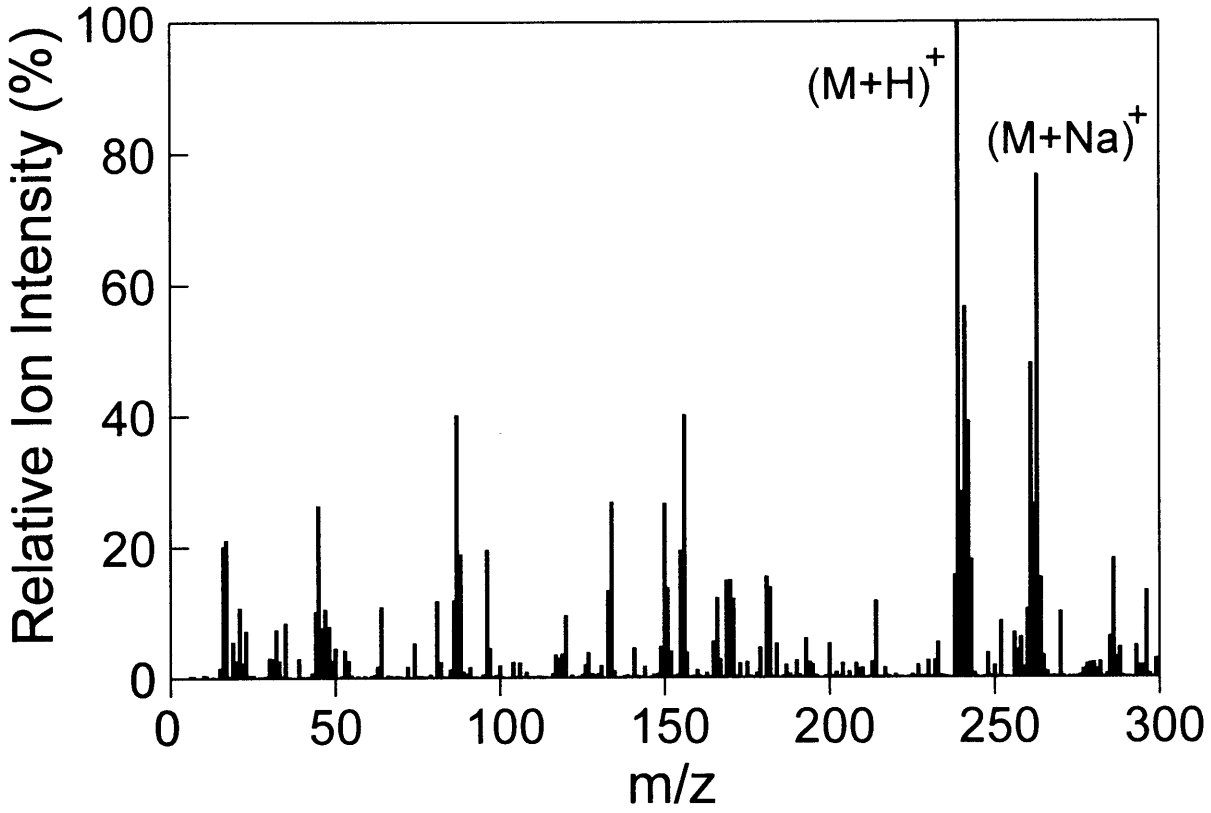

Figure 4.6 Mass spectrum obtained from a 50:50 methanol/water solution of thiuram by the SSI technique. The solution concentration was 10 μM.

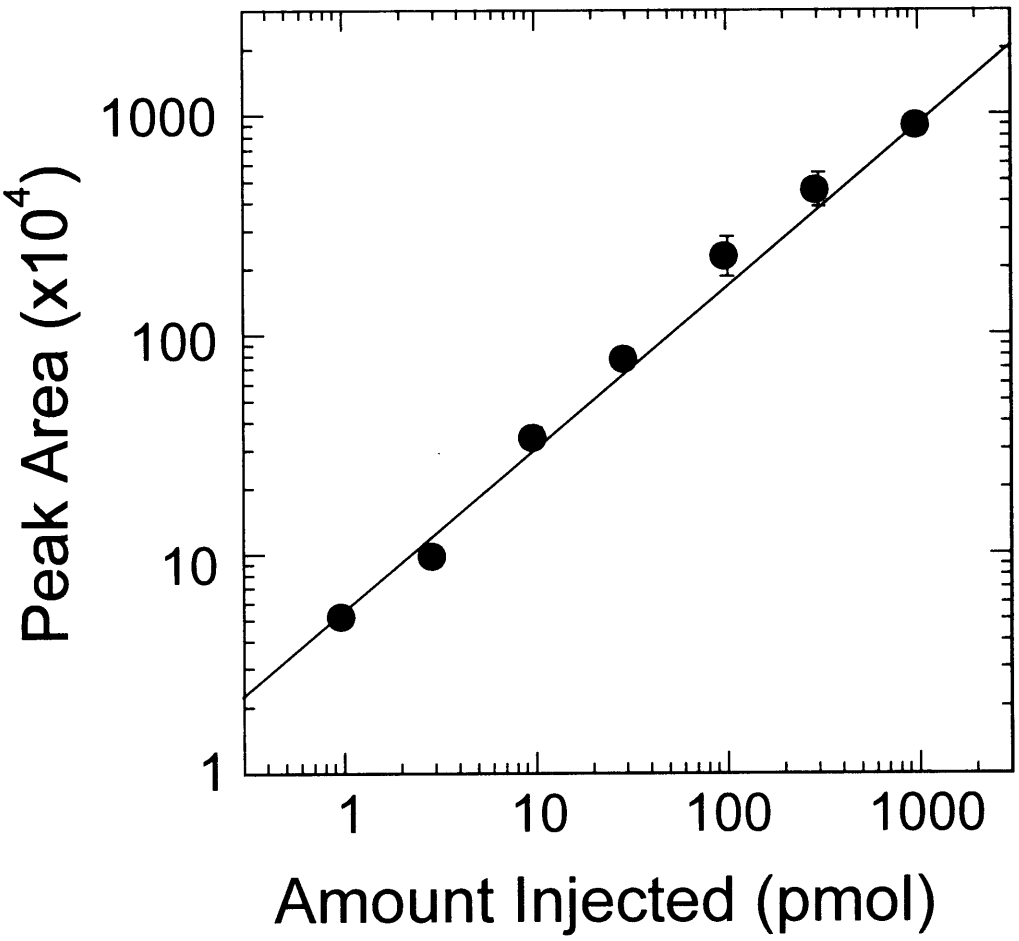

Figure 4.7 Calibration curve for the protonated simazine molecule (m/z = 202).

Figure 4.8 shows the analysis result for a mixture of pesticides when semi-micro LC/SSI-MS with the multiple ion detection (MID) mode was used. Simazine, thiuram, and thiobencarb were used in this measurement and each injected component was 50 pmol. To separate these compounds, the mobile phase was 60% water/40% methanol for the first ten minutes, then a linear gradient from 60% water/40% methanol to 100% methanol over ten minutes was used. The flow rate of the mobile phase was set at 200 μL/min. Since intense protonated molecules can be obtained for these compounds, the ions were monitored at an m/z of 202, 241, and 258 for simazine, thiuram, and thiobencarb, respectively. Peaks for each m/z were clearly observed at 15, 14, and 37 min, respectively. A smaller peak corresponding to protonated molecules of thiuram combined with water molecules was observed at 14 minutes (m/z = 258).

These results demonstrate that semi-micro LC/SSI-MS is a very powerful tool for pesticide analysis.

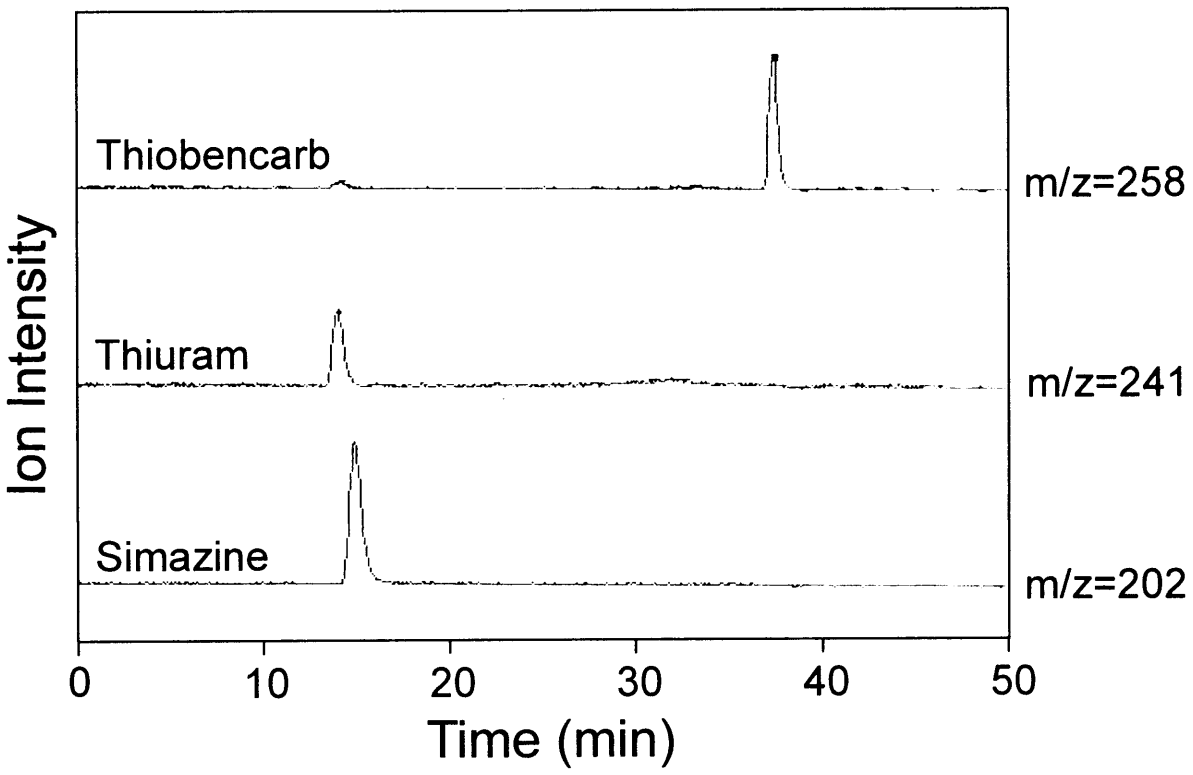

Figure 4.8 On-line LC/MS analysis of a three-pesticide mixture (simazine, thiuram, and thiobencarb). Each component represents 50 pmol.

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5. Improvement of the Sonic Spray Interface for High-flow Rates

The author improved the sonic spray interface to enable the analysis of multiply-charged ions of protein from a solution at a flow rate of 1 mL/min using a conventional liquid chromatograph / mass spectrometer (LC/MS). This was done by adding a multi-hole plate in front of the sampling orifice of the mass spectrometer. The plate did not have a hole coaxial to the sampling orifice, but had small holes around the central region of the plate. This plate reduced the density of the solvent molecules in the sprayed gas introduced into the vacuum region through the sampling orifice from the atmosphere, and prevented the ions from being solvated and becoming charged droplets due to the cooling that follows adiabatic expansion of the sprayed gas. With this improvement, multiply-charged ions whose charge distribution ranged from 11+ to 16+ were analyzed from a 1-μM cytochrome-c solution at a high solution-flow rate of 1 mL/min without using a splitter.

5.1 Introduction

Liquid chromatography / mass spectrometry (LC/MS) is a powerful tool for analyzing mixtures in a solution in various fields of science. Spray ionization techniques such as atmospheric pressure chemical ionization (APCI) [1,2], electrospray ionization (ESI) [3-5], ion spray (IS) [6], and atmospheric pressure spray (APS) [7,8] have been developed to provide an LC/MS interface. Of these techniques, only ESI and IS can produce multiply-charged ions of large biomolecules such as proteins. ESI without an assistant nebulizing gas is most effective at solution-flow rates below 15 μL/min [9]. However, the size of droplets produced by ESI increases with an increasing solution-flow rate. For example, the mean diameter of water droplets produced at flow rates ranging from 3 to 39.4 μL/min is 1.4 to 10 μm [10]. In the IS technique, use of solution-flow rates ranging from a few microliters per minute to 2 mL/min is enabled by a liquid shield [11]. Since the electric field of each droplet is not high enough to desorb ions at a high solution-flow rate, the liquid shield reduces the droplet size through impact to aid desolvation. Nevertheless, when the solution flow rate is about 1 mL/min, the detection of multiply-charged ions with over 10 charges, without use of a splitter, has not been not reported.

In the sonic spray ionization (SSI) interface for LC/MS [12-15], the range of solution flow rates is under 0.2 mL/min, making SSI a useful interface for semi-micro LC/MS [15]. However, it is difficult to use SSI as an interface for conventional LC/MS, in which the solution-flow rate is much higher, i.e., above 1 mL/min.

In SSI, the droplets produced by the high-speed gas flow are very small, i.e. about 1 μm in diameter and the size is almost independent of the solution-flow rate [16]. Thus,

they evaporate quickly. Also, the ions produced in a sprayed gas containing a high density of solvent molecules at high-solution flow rates in the atmosphere associate with gas-phase solvent molecules, forming large clusters and charged droplets due to adiabatic expansion when they are introduced into the vacuum region through the sampling orifice of the mass spectrometer. Since multiply-charged ions tend to be more solvated than singly-charged ions, the intensity of multiply-charged ions is considerably reduced. The author modified the SSI interface to reduce the density of solvent molecules in the sprayed gas before they are introduced into the vacuum region, and this has made it possible to analyze a solution -- without using a splitter -- over a wide range of solution-flow rates, including the higher solution-flow rates used in conventional LC/MS.

5.2 Experimental Section

The experimental setup was almost the same as the previous interface [12-15,17], except that the author added a multi-hole plate. Figure 5.1 shows cross-sectional views of the improved SSI interface and the multi-hole plate. The flow rate of the nitrogen gas in the standard state was 3-4 L/min. (Only for semi-micro LC/MS without the multi-hole plate is the higher flow rate previously used necessary.) A voltage of -1.2 kV was applied to the orifice and the source housing to increase the charge density of droplets produced by the gas flow, thus increasing the efficiency of positive-ion formation [13, section 2.2].

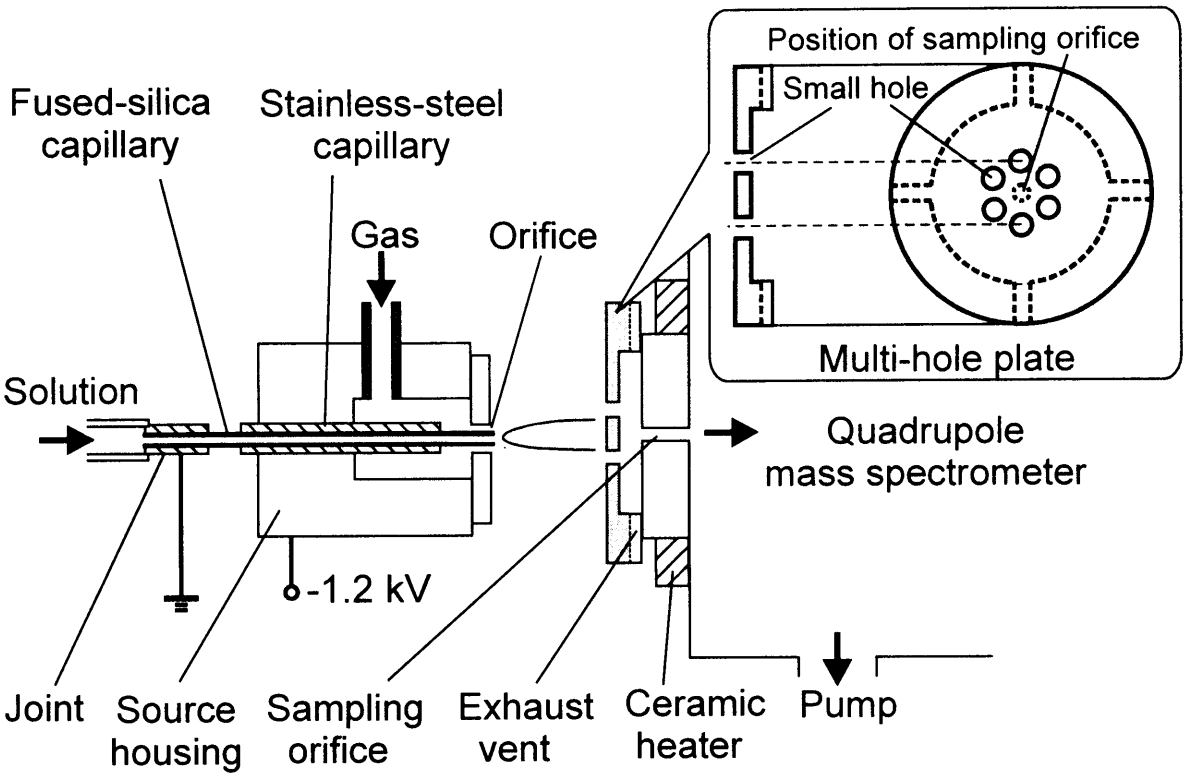

Figure 5.1 Cross-sectional views of the improved sonic spray interface and the multi-hole plate.

In the previous interface, the stainless-steel plate to prevent cooling of the sampling orifice due to gas flow and droplet evaporation was set in front of the sampling orifice 3 mm from the capillary tip, and the plate had a 2-mm-diameter hole coaxial to the sampling orifice in the center of the plate [13, 15, section 3.2]. In the improved interface, the multi-hole plate did not have a hole coaxial to the sampling orifice of the mass spectrometer, but had small 0.8-mm-diameter holes around the central region of the plate. It also has exhaust vents to prevent a build-up of pressure, and thus an increased density of solvent molecules, between the plate and the sampling orifice. The diagonal distance between the small holes was about 4 mm. The central axis of the capillary was aligned with that of the sampling orifice but not with that of the small holes, so the sprayed gas struck the central region of the plate and was diffused -- especially, the center of the spray corn where the density of solvent molecules is highest. The ions and charged droplets produced in the atmosphere passed through the small holes in the plate, then were introduced into the vacuum region of the mass spectrometer. (The details of the mass spectrometer have been described elsewhere [15,17].) An electrostatic ion guide [18] was set in front of a quadrupole mass analyzer in the mass analyzing region.

5.3 Results and Discussion

To determine a suitable diagonal distance between the small holes of the multi-hole plate, the author measured the ion intensity of lysine and gramicidin-S using multi-hole plates with a diagonal distance of 2, 3, 4, or 5 mm. The dependence of the ion intensity of the protonated lysine molecule (m/z = 147) and the doubly-protonated gramicidin-S molecule (m/z= 571) on the diagonal distance at solution-flow rates of 1, 0.2, and 0.03 mL/min is shown in Fig. 5.2. The lysine and gramicidin-S solutions were 50:50 methanol/water solutions with a concentration of 1 μM. Since the ion intensity at solution-flow rates of 1 and 0.2 mL/min for the lysine ion (Fig. 5.2(a)) and 0.2 and 0.03 mL/min for the gramicidin-S ion (Fig. 5.2(b)) reached a maximum at a diagonal distance of 4 mm, this distance was used as the standard distance for the multi-hole plate.

When using the multi-hole plate, a high gas flow with a velocity above the sonic velocity was not needed to obtain intense ions even though the solution-flow rate was over 0.1 mL/min. Figure 5.3 shows the dependence of the ion intensity of doubly-protonated gramicidin-S (as was used in the chapter 2 experiment) on the gas flow rate with the multi-hole plate at a solution-flow rate of 1 mL/min. The ion intensity reached a maximum at about 3 L/min, which was almost the same at low solution-flow rates, i.e., 0.03 mL/min, since the density of solvent molecules in the sprayed gas was adequately decreased by diffusion of the sprayed gas due to the multi-hole plate.

Mass spectra obtained from a lysine solution at a solution-flow rate of 1 mL/min using the previous interface and when using the multi-hole plate are shown in Fig. 5.4. The lysine solution was a 50:50 methanol/water solution at a concentration of 1 μM. The

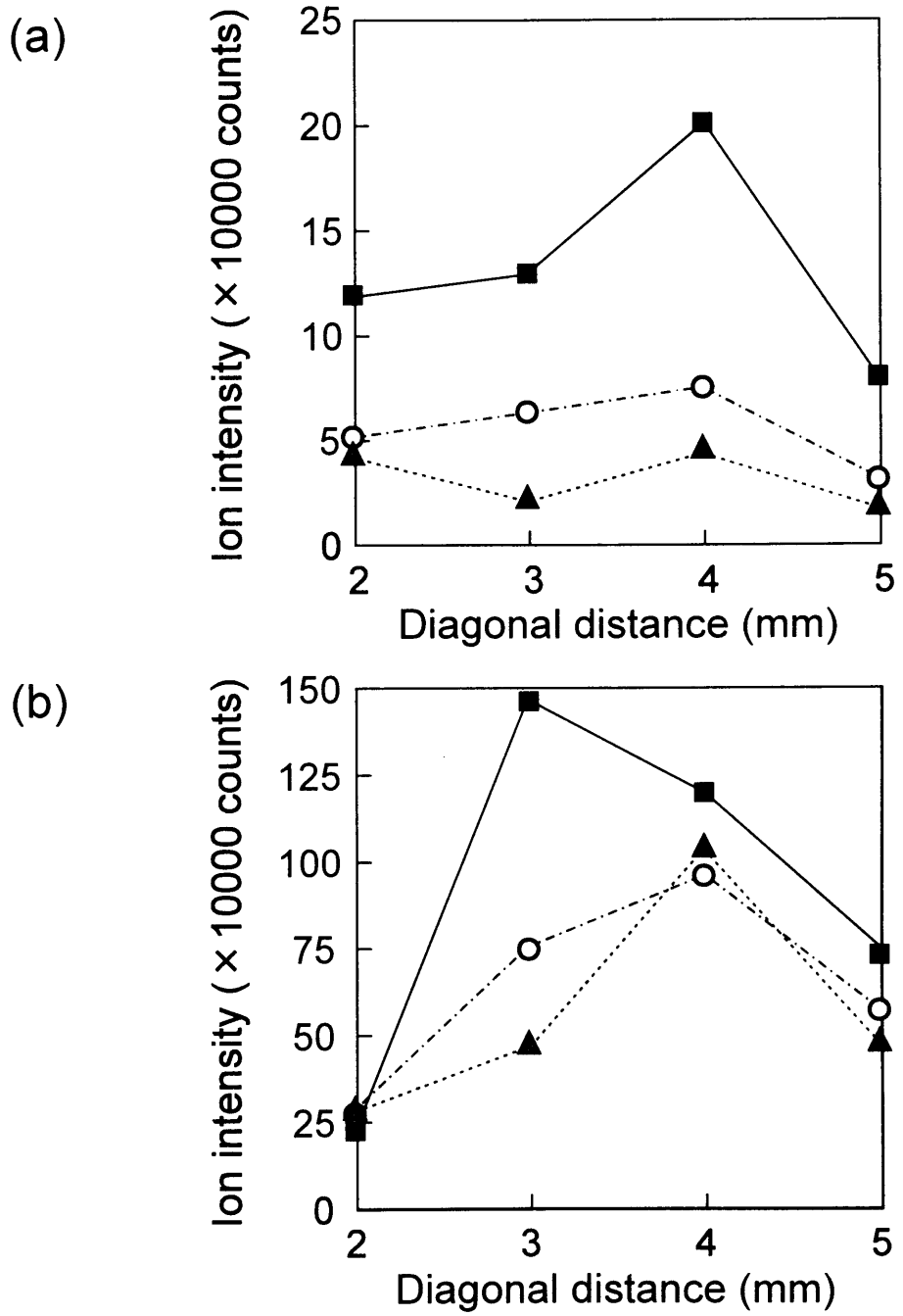

Figure 5.2 Relationship between the ion intensity of (a) the protonated lysine molecule (m/z = 147), (b) the doubly-protonated gramicidin-S molecule (m/z = 571) and the diagonal distance between the small holes of the multi-hole plate at solution-flow rates of 1 (■), 0.2 (O), and 0.03 (▲) mL/min. The solution concentrations were 1 μM. The distance between the capillary tip and the surface of the plate was 3 mm.

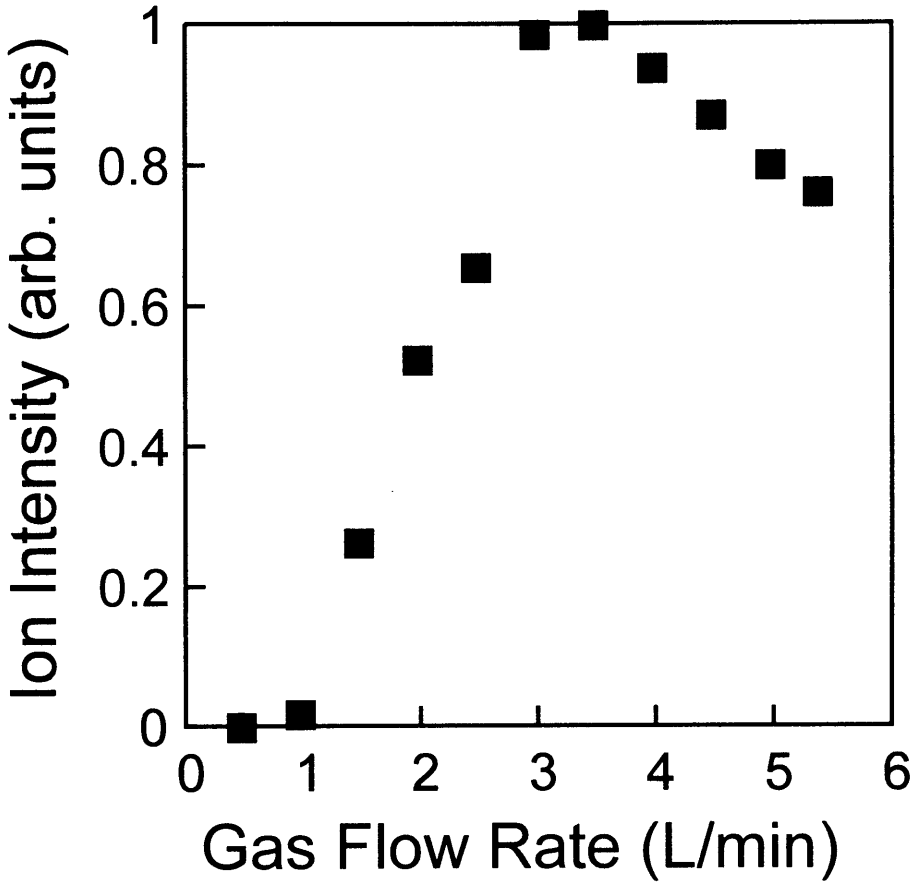

Figure 5.3 Ion intensity of doubly-protonated gramicidin-S molecules (m/z = 571) from a 1-μM solution as a function of gas flow rate when using the multi-hole ptate. The solution-flow rate was 1 mL/min.

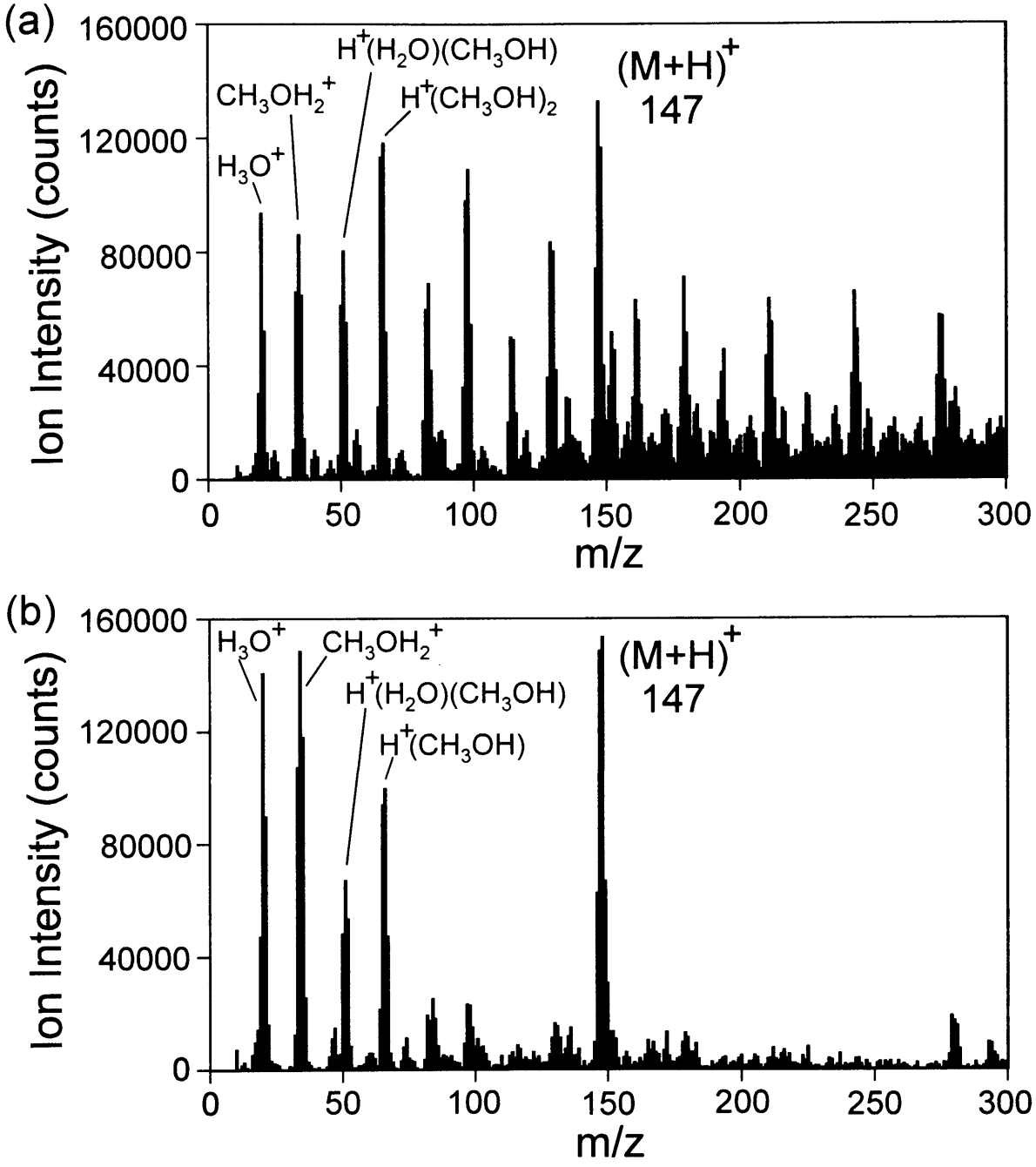

Figure 5.4 Mass spectra from a 50:50 methanol/water solution of lysine obtained using (a) the previous, and (b) the improved sonic spray interface at a solution-flow rate of 1 mL/min. The solution concentration was 1 μM.

protonated molecules of lysine were detected at m/z = 147 with the previous interface, but clusters of methanol and water molecules were also detected. Furthermore, a lot of random noise was observed over the entire spectrum. This noise was caused by detecting charged droplets whose m/z values were too high to be affected by the electric field of the quadrupole mass spectrometer. Due to the high noise level of this spectrum, the signal-to-noise ratio was about seven.

With the multi-hole plate, the protonated molecules of lysine were still detected at m/z = 147, but at a higher intensity. Clusters of methanol and water molecules (such as $H^+(H_2O)(CH_3OH)$ and $H^+(CH_3OH)_2$) were observed, but only under m/z = 100. Also, the noise level was lower than with the previous interface; the signal-to-noise ratio of the improved interface was about twenty-seven -- almost four times that of the previous interface. Therefore, the multi-hole plate enables SSI at high solution-flow rates of up to 1 mL/min or higher.

When the sprayed gas is introduced into the vacuum region of the mass spectrometer through the sampling orifice, solvent molecules in the sprayed gas are cooled by adiabatic expansion and tend to associate with each other to form clusters and droplets. This significantly reduces the sensitivity of the instrument. At high solution-flow rates, the sprayed gas contains a high density of solvent molecules, so this tendency is particularly noticeable. Sample ions in the sprayed gas are also cooled and tend to associate with the solvent molecules to form clusters and charged droplets in the mass spectrometer, reducing the ion intensity at high solution-flow rates. Multiply-charged ions are more likely to become charged droplets than singly-charged ions, because the Gibbs energy for solvation increases with an increasing charge number of positive analyte ion. To prevent reduced ion intensity and increased noise, the density of solvent molecules in the sprayed gas must be

reduced before the molecules pass into the vacuum region through the sampling orifice. In the improved SSI interface, the density of solvent molecules is diluted by diffusion of the sprayed gas when it strikes the multi-hole plate. Then the sprayed gas passes into the small space between the plate and the sampling orifice through the small holes in the plate, and the density of solvent molecules is further reduced. Therefore, a sprayed gas that contains a suitable density of solvent molecules, in which the tendency towards association is inhibited, is introduced into the vacuum region through the sampling orifice.

Typical mass spectra of gramicidin-S measured using the improved interface are shown in Fig. 5.5. The gramicidin-S solution was a 50:50 methanol/water solution at a concentration of 1 μM. The spectra were obtained at flow rates of 1, 0.2, and 0.03 mL/min. In these spectra, singly- and doubly- protonated molecules of gramicidin-S, respectively, were detected at m/z = 1141 and 571. The ion intensities of the doubly-protonated gramicidin-S molecules (m/z = 571) were almost at the same level with good sensitivity at all solution flow rates.

Furthermore, the author has demonstrated that multiply-charged ions can be analyzed with the improved SSI interface from a 1-μM cytochrome-c (from a sheep heart) solution in water/methanol/acetic acid (47.5/47.5/5%, v/v/v) at 1 mL/min (Fig. 5.6). The charge distribution ranged from 11+ to 16+ while the base peak at m/z = 947 corresponded to $(M+13H)^{13+}$. The ion intensity of cytochrome-c at 1 mL/min was comparable to that detected at 0.03 mL/min using the original SSI interface (data is shown in chapter 2), though there was a two- or three-charge reduction compared to the ions produced at 0.03 mL/min. This can be ascribed to gas-phase proton transfer reactions between the multiply-charged ions and solvent molecules.

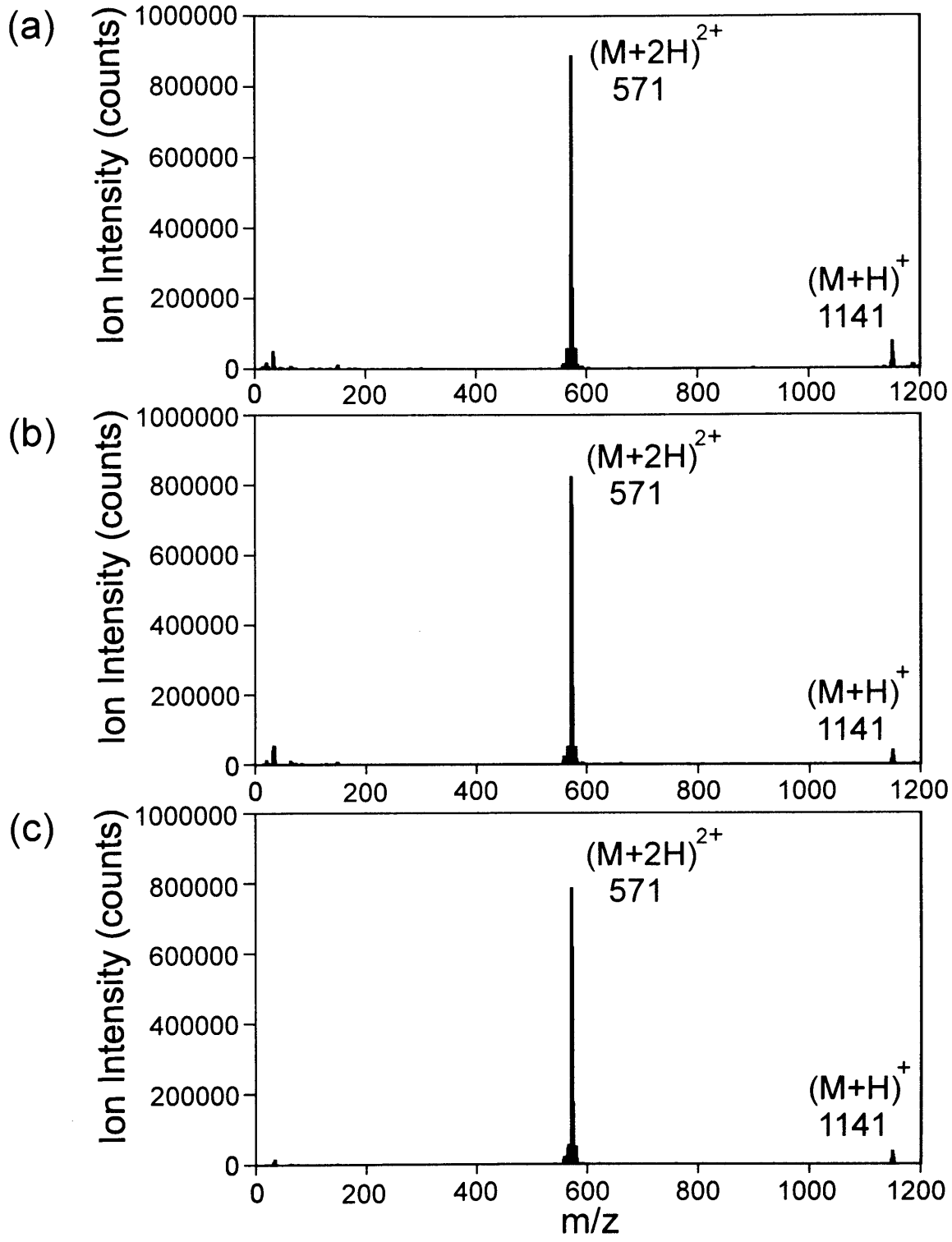

Figure 5.5 Mass spectra from a 50:50 methanol/water solution of gramicidin-S obtained using the improved sonic spray interface at a solution-flow rate of (a) 1 mL/min, (b) 0.2 mL/min, and (c) 0.03 mL/min. The solution concentration was 1 μM.

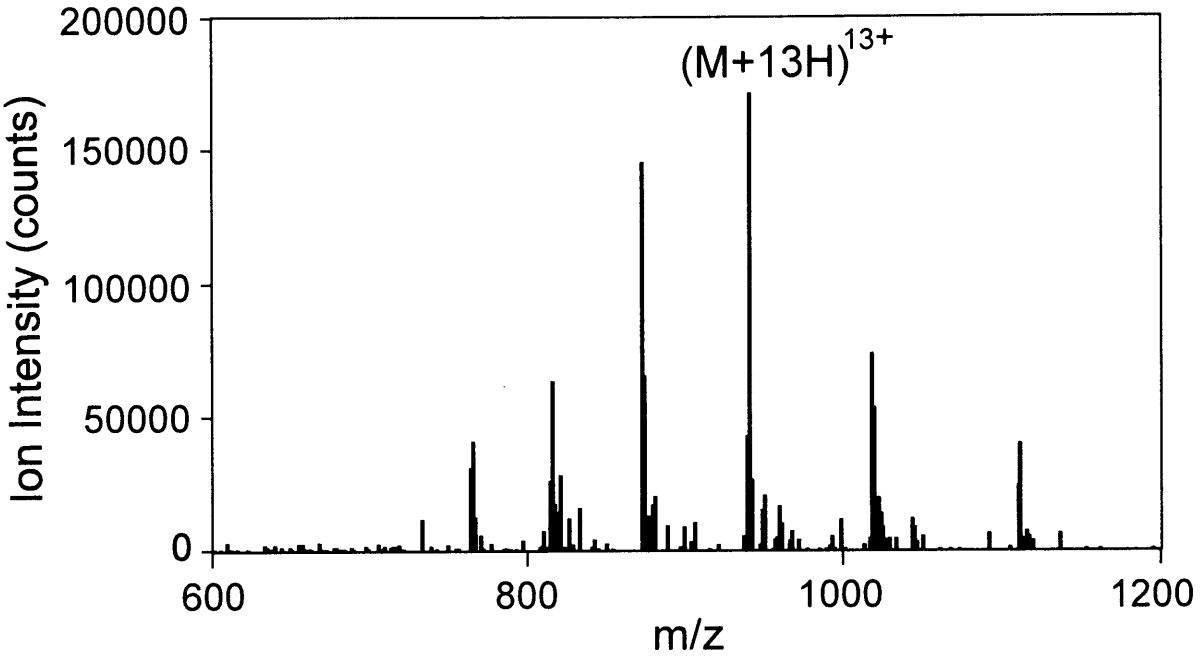

Figure 5.6 Mass spectrum from a 47.5:47.5:5 methanol/water/acetic acid solution of cytochrome-c obtained using the improved sonic spray interface at a solution-flow rate of 1 mL/min. The solution concentration was 1 μM.

With this improvement, SSI can be used over a wide range of solution-flow rates. This enables the analysis of highly-charged ions of proteins at various solution-flow rates using simple systems without a splitter.

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6. A Sonic Spray Interface for Capillary Electrophoresis / Mass Spectrometry

The author has also developed a sonic spray ionization interface that enables coupling of capillary electrophoresis (CE) and mass spectrometry (MS). In sonic spray ionization, the sample solution can be sprayed at any typically used solution-flow rate from the sample-introduction capillary with a high-speed gas flow. Therefore, SSI can be used with a wide range of buffer solutions regardless of the conductivity of the solutions. However, the pressure around the tip of the sample-introduction capillary is reduced by the high-speed gas flow, so the solution is pumped into the capillary at a flow rate above 0.1 μL/min due to the difference of pressure between the two ends of the capillary. Since the solution-flow rate in CE is much lower than this pumping rate, the resolution of CE separation is likely to be decreased by the pumping effect when an electrophoresis capillary is connected directly with the sample-introduction capillary. To avoid this in the CE/MS interface, the author added a buffer reservoir between the sample-introduction capillary of the interface and the electrophoresis capillary, and confirmed that this prevented pumping of the solution in the electrophoresis capillary due to the pressure difference. The author also

demonstrated CE/MS analysis with a mobile-phase buffer containing 15 mM of phosphate by filling the buffer reservoir with an acetic-acid solution as a substitute for the mobile-phase buffer. This increased the ion intensity one-hundred fold by enhancing the evaporation of charged droplets produced by the spray.

6.1 Introduction

Capillary electrophoresis (CE) is an extremely efficient tool for separating mixtures because of its high-resolution separation capability. However, the ability to identify chemical species with only CE plus a single-parameter detector such as ultraviolet absorption is limited because the migration time is apt to shift, so use of a mass spectrometer as a CE detector is useful. Together, capillary electrophoresis and mass spectrometry (CE/MS) [1] will be an important tool in various fields of science. The coupling of CE and MS by electrospray ionization (ESI) [1-5], ion spray (IS) technique [6], and a continuous-flow fast-atom bombardment (CF-FAB) technique [7,8], have been described. In the ESI techniques, coupling of CE and MS through use of a liquid sheath [2,3], a sheathless interface [1,4], and a microdialysis junction [5], have been reported. The liquid junction [6] is used to deliver a make-up flow when coupling by IS, and also in the CF-FAB interface technique [8]. However, CE/MS analysis has had to be performed using a limited selection of mobile-phase buffers, because the use of nonvolatile buffers is

generally avoided. Although phosphates are widely used as a mobile-phase buffer for capillary electrophoresis, their usable concentrations in CE/MS are limited because sprays of high-conductivity solutions such as phosphate buffers are unstable in ESI and IS.

The SSI interface developed by the author for use in semi-micro and conventional LC/MS [12,13] produces sprays that are stable under all typically used solution-flow rates and buffer conditions, because sprays are generated only by gas flow. However the pressure around the tip of the sample-introduction capillary is reduced by the high-speed gas flow, so the solution is pumped into the capillary due to the difference of pressure between the two ends of the capillary. Since the pumping rate exceeds the CE solution-flow rate, the resolution of CE separation is likely to be decreased, even though ions are readily formed at any solution-flow rate. To prevent the solution in the electrophoresis capillary being pumped, the author has developed an SSI interface specifically for CE/MS.

6.2 Estimation of Pumping Rates

The author began by estimating pumping rates into the capillary due to the difference of pressure between the two ends of the capillary caused by the high-speed gas flow. Generally, the pumping rate of a solution (Q) due to a pressure difference between two ends of a capillary is described by the Hargen-Poiseuille equation:

$$Q = \Delta p \, \pi r^4/(8\mu L) \qquad (6.1)$$

In Eq. (6.1), Δp is the pressure difference between the two ends of the capillary, r is the capillary radius, μ is the solution viscosity, and L is the capillary length. The pumping rates for any capillary radii can be estimated if Δp is determined when the solution is pumped by a high-speed gas flow around the capillary tip. The author obtained the value of Δp, when using 60-cm-long capillaries with a radius of 25, 35.5, or 50 μm, by measuring the pumping rates of a 50% methanol solution in water in these capillaries at a sonic-velocity gas-flow rate. The μ value of the 50% methanol solution in water at room temperature was approximately 2×10^{-3} Pa/s [14]; accordingly Δp was about 0.2×10^5 Pa. Using this value, the dependence of the pumping rate on the inner diameter of a 60-cm-long capillary was estimated (Fig. 6.1). The pumping rate decreased with a decreasing inner diameter, and the pumping rates of capillaries with an inner diameter of less than 10 μm were lower than the flow rates due to electroosmosis (which range from 1 nL/min to 0.1 μL/min). On the other hand, capillaries with an inner diameter of over 50 μm are the most practical to use because it is difficult to inject a sample into a capillary with a smaller inner diameter. Therefore, the author used a 50-μm-inner-diameter capillary, in which the pumping rate is significant.

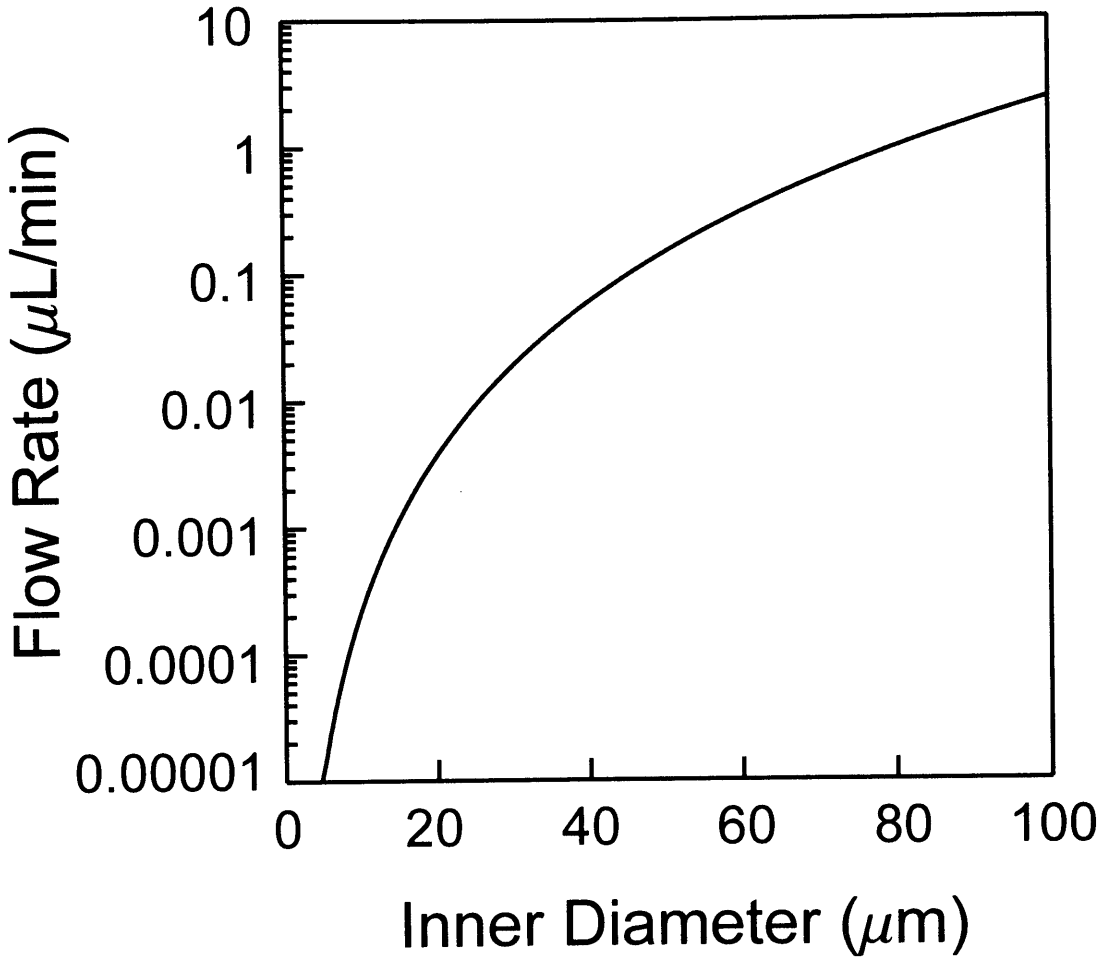

Figure 6.1 The dependence of the pumping rate on the inner diameter of a 60-cm-long capillary.

6.3 Experimental Section

Figure 6.2 is a cross-sectional view of the SSI interface for CE/MS. One end of the fused-silica sample-introduction capillary (50-μm i.d., 150-μm o.d., 10 cm long, GL Science, Tokyo) was inserted into the orifice (0.28-mm i.d.) in the duralumin source housing. The flow rate of the nitrogen gas at the standard state was 2 L/min, which was lower than in the previous experiments since the orifice was smaller in this experiment. A voltage of -1.2 kV was applied to the orifice and the source housing. (The details of the mass spectrometer are described in chapter 3 and elsewhere [12,15].)

The other end of the sample-introduction capillary was inserted into a Teflon tube that passed through the buffer reservoir. The buffer reservoir was filled with a buffer solution so that the pinhole (whose diameter was about 0.1 mm) in the Teflon tube was submerged and the buffer solution flowed through the pinhole into the sample-introduction capillary by the pumping effect. The electrical potential of the buffer solution in the buffer reservoir was held at ground potential through a platinum electrode. A fused-silica capillary for electrophoresis (50-μm i.d., 150-μm o.d., GL Science, Tokyo) was inserted into the Teflon tube from the opposite end. The capillaries were set opposite to each other near the pinhole in the Teflon tube. The other end of the electrophoresis capillary was put in a mobile-phase reservoir filled with the buffer solution. A high voltage was applied between the buffer reservoir and the mobile-phase reservoir to generate the electrophoresis. The sample solution separated by the electrophoresis was mixed with the buffer solution in the area beneath the pinhole in the Teflon tube, and pumped into the sample-introduction capillary. Since the pumping rate of the 10-cm-long capillary was about 1 μL/min, as estimated from

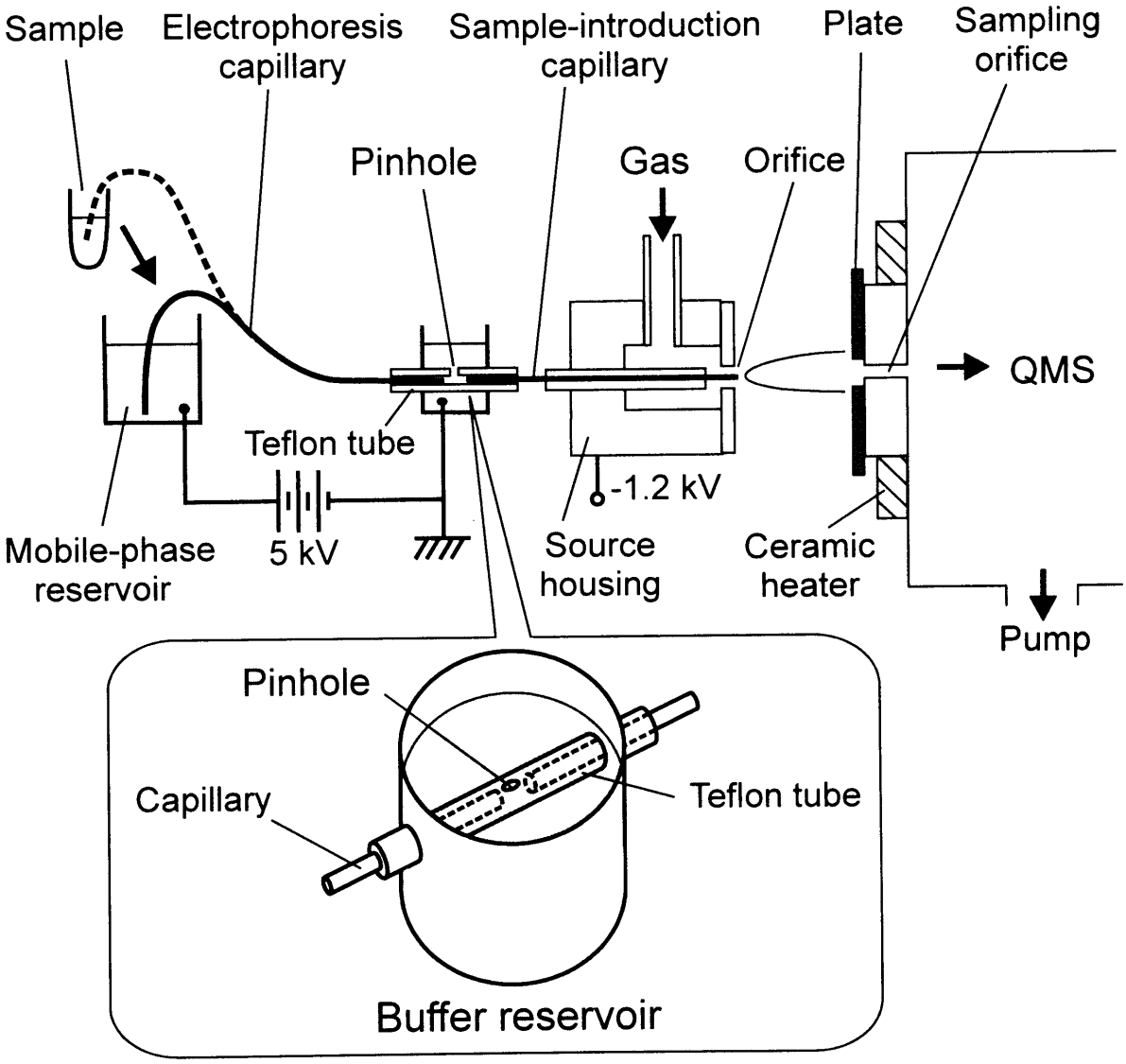

Figure 6.2 Cross-sectional view of the sonic spray interface for CE/MS, with a close-up of the buffer reservoir.

Eq. (6.1), the solution from the buffer reservoir was pumped into the sample-introduction capillary at a flow rate more than ten times as high as the electroosmosis flow rate. Therefore, the sample solution did not diffuse into the buffer reservoir. The sample solution was then sprayed from the sample-introduction capillary by the sonic gas flow.

In this interface, since both capillaries are set opposite to each other in the Teflon tube, alignment of the capillaries is very easy. Furthermore, the electrophoresis capillary can be easily changed and the inner diameter of the sample-introduction capillary can differ from that of the electrophoresis capillary.

6.4 Results and Discussion

A Sonic Spray Interface for CE/MS

When no voltage was applied between the two ends of the electrophoresis capillary, ions of the sample introduced into the electrophoresis capillary could not be observed (Fig. 6.3). In this experiment, the electrophoresis capillary and the mobile-phase reservoir were filled with the sample solution (1-μM gramicidin-S and 15-mM ammonium acetate in a 50:50 water/methanol solution, pH 6.3), and the buffer reservoir was filled with the buffer solution (15-mM ammonium acetate in a 50:50 water/methanol solution, pH 6.3). When 5 kV of voltage was applied between the buffer reservoir and the mobile-phase reservoir (denoted by arrow A in Fig. 6.3), the sample solution flowed by electroosmosis and sample

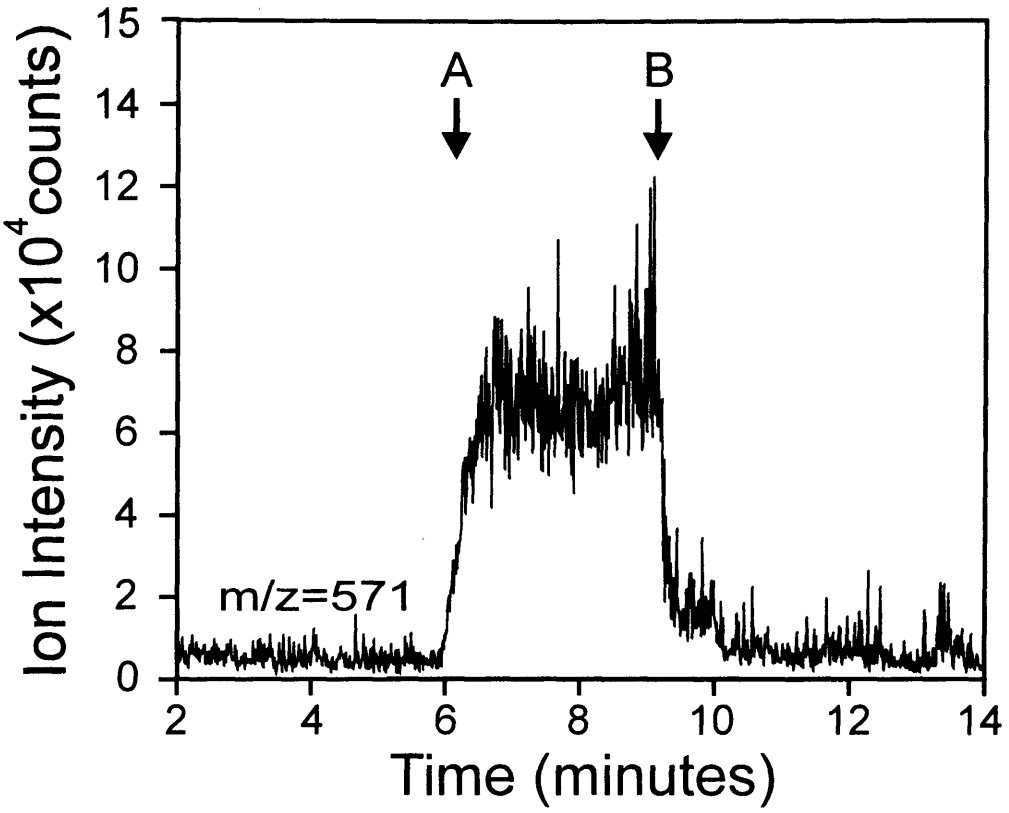

Figure 6.3 Electrophoretogram of gramicidin-S. The electrophoresis capillary and the mobile-phase reservoir were filled with the sample solution (1-μM gramicidin-S and 15-mM ammonium acetate in a 50:50 water/methanol solution, pH 6.3), and the buffer reservoir was filled with the buffer solution (15 mM ammonium acetate in a 50:50 water/methanol solution, pH 6.3). When a voltage was applied between the buffer reservoir and the mobile-phase reservoir (A), the sample ions were detected. When the voltage was turned off (B), the ions were not detected, even though gas was flowing around the capillary tip.

ions were detected. When the voltage was turned off (point B), ions were not detected, even though gas continued to flow around the capillary tip. Therefore, the solution in the electrophoresis capillary was not being pumped by the gas flow. Thus, this interface can be used for CE/MS without any loss of resolution.

Figure 6.4 is an electrophoretogram of a dopamine and GABA mixture obtained using this interface. Both reservoirs were filled with a 15-mM ammonium acetate buffer (pH 6.3) in water/methanol (50/50%, v/v), and 12 and 15 pmol of dopamine and GABA, respectively, were injected into the electrophoresis capillary (40 cm long) by the gravity method. A voltage of 5 kV was applied between the two reservoirs. The ion intensity monitored at m/z = 104 and 154. After 13 and 18 minutes, respectively, peaks corresponding to the dopamine and GABA ions were observed in the electrophoretogram. The GABA and dopamine were clearly separated and detected. Separation efficiencies for the GABA and dopamine were about 10000 and 30000 theoretical plates, respectively.

CE/MS analysis using a phosphate buffer

With SSI, any kind of solution are sprayed steadily, because the solutions are sprayed only by the high-speed gas flow. However, the ion intensity of a sample solution containing phosphate is lower than that of a non-phosphate sample solution because phosphates are nonvolatile. Therefore, the solvent molecules are less likely to evaporate and the ionization efficiency is low. In this case, adding a volatile substance such as acetic

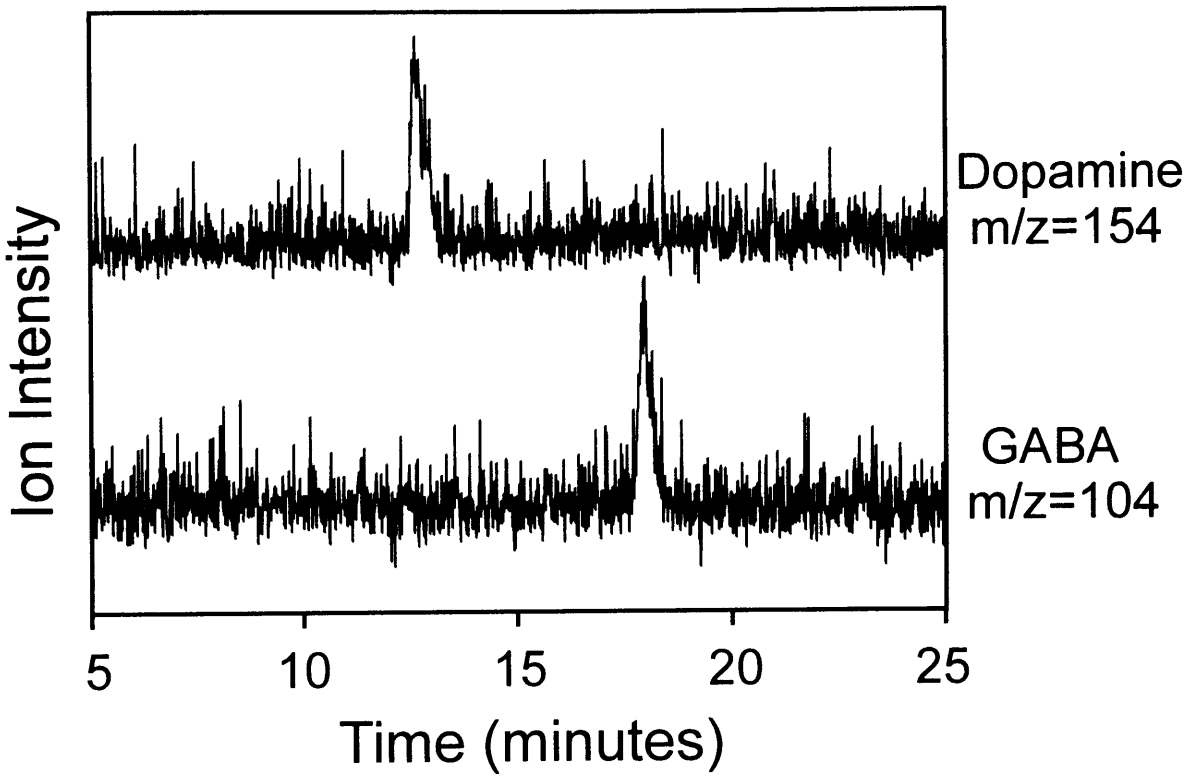

Figure 6.4 Electrophoretogram of dopamine and GABA. Both reservoirs were filled with a 15-mM ammonium acetate buffer (pH 6.3) in water/methanol (50/50%, v/v), and the injected samples were 12 and 15 pmol of dopamine and GABA, respectively. The electrophoresis capillary was 40 cm long and 5 kV was applied between the two reservoirs.

acid increases the ion intensity. Figure 6.5 shows electrophoretograms of gramicidin-S, where 2 pmol of gramicidin-S was introduced into the electrophoresis capillary (40 cm long) by the gravity method. The buffer reservoir and the mobile-phase reservoir were filled with either a 15-mM phosphate buffer (pH 6.3) in water/methanol (50/50%, v/v) (Fig. 6.5(a)), or a 15-mM phosphate buffer and an acetic-acid solution (42/50/8% water/methanol/acetic acid, pH 1.4) (Fig. 6.5(b)). The signal-to-noise ratio of the Gramicidin-S peak in Fig. 6.5(b) was about one-hundred times as high as in Fig. 6.5(a) because of the accelerated solvent evaporation due to the volatile substance added to the solution. Although a volatile buffer can also be added with the ESI interface by using a liquid sheath [2, 3], it is probably difficult to ensure sufficient mixing because of the short residence time in the cone [2]. Since the residence time of this interface is much longer than that with the liquid sheath, the ionization efficiency is significantly increased. This result indicates that high-concentration phosphate buffers of above 15 mM can be used in this interface.

Figure 6.6 is an electrophoretogram of the dopamine and GABA mixture. In this case, the mobile-phase reservoir was filled with a 15-mM phosphate buffer (pH 6.3) in water/methanol (50/50%, v/v), and the buffer reservoir was filled with an acetic-acid solution (42/50/8% water/methanol/acetic acid, pH 1.4). The author injected 12 pmol of dopamine and 15 pmol of GABA and 5 kV was applied between the two reservoirs then monitored the intensities at m/z = 104 and 154. After 17 and 20 minutes, respectively, peaks corresponding to the dopamine and GABA ions were observed. The signal-to-noise ratios of the GABA and dopamine peaks in Fig. 6.6 roughly two to four times as high as those in Fig. 6.4. Also, the peak width for GABA was much smaller than that for dopamine

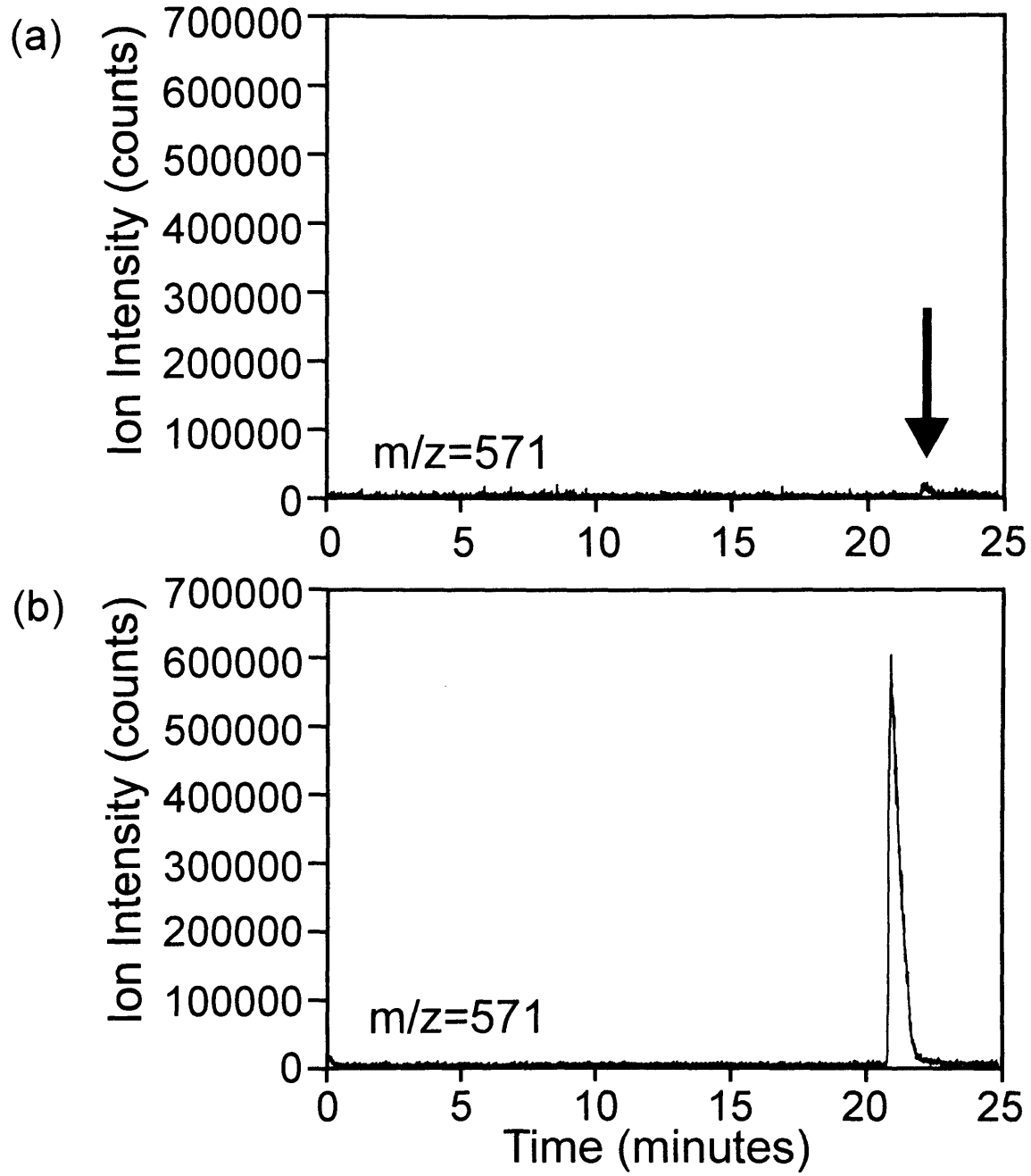

Figure 6.5 Electrophoretograms of Gramicidin-S. The buffer reservoir and the mobile-phase reservoir were filled with (a) a 15-mM phosphate buffer (pH 6.3) in water/methanol (50/50%, v/v), or (b) a 15-mM phosphate buffer and an acetic-acid solution (42/50/8% water/methanol/acetic acid, pH 1.4). The injected sample was 2 pmol. The electrophoresis capillary was 40 cm long and 5 kV was applied between the two reservoirs.

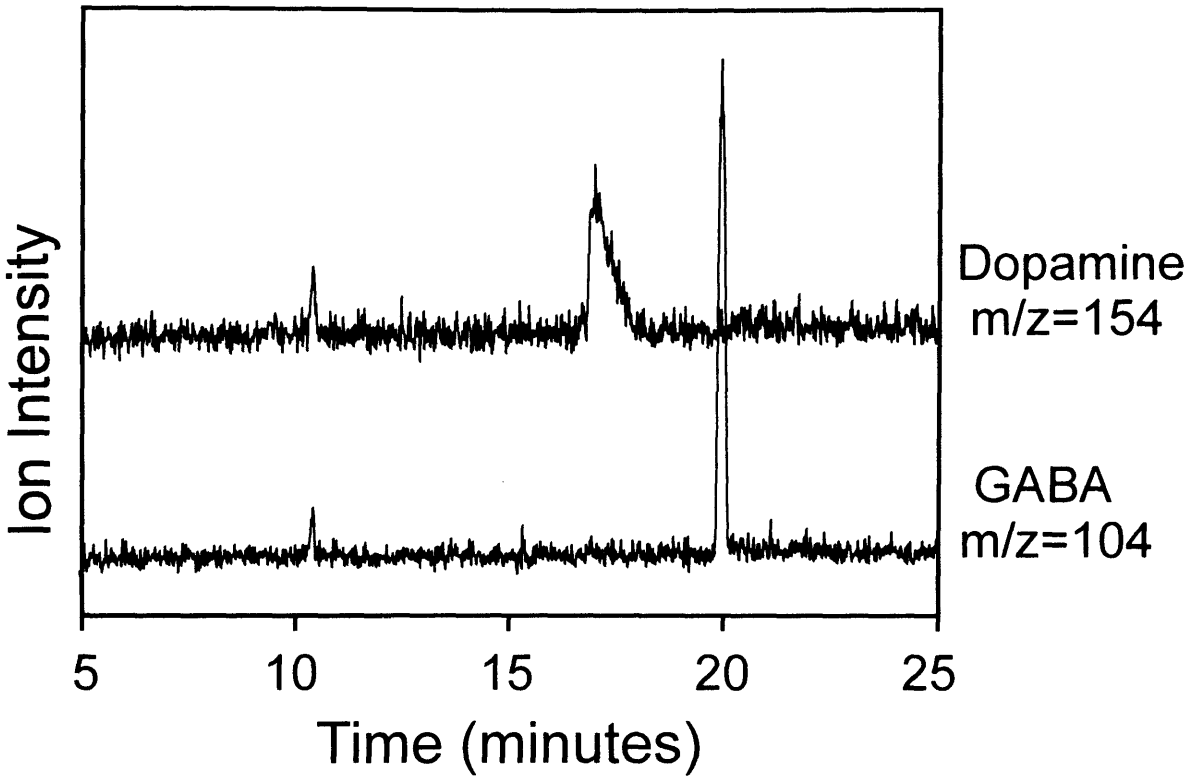

Figure 6.6 Electrophoretogram of dopamine and GABA. The buffer reservoir and the mobile-phase reservoir were filled with a 15-mM phosphate buffer (pH 6.3) in water/methanol (50/50%, v/v) and an acetic-acid solution (42/50/8% water/methanol/acetic acid, pH 1.4). The injected samples were 12 and 15 pmol of dopamine and GABA, respectively. The electrophoresis capillary was 40 cm long and 5 kV was applied between the two reservoirs.

in Fig. 6.6. This suggests that GABA, whose isoelectric pH was about 4, was concentrated by the pH gradient ranging from 1.4 to 6.3 in the electrophoresis capillary.

In conclusion, the SSI interface enables CE/MS analysis under virtually any conditions of separation. Furthermore, since any kind of solution can be used in the buffer reservoir, CE/SSI-MS provides a powerful tool for analysis.

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CONCLUSION

In this study, the author has developed techniques to couple a separation tool, e.g. a semi-micro liquid chromatograph (LC), a conventional liquid chromatograph, or capillary electrophoresis (CE), with a mass spectrometer (MS) using sonic spray ionization (SSI) as an interface. The efficiency of negative-ion formation in sonic spray ionization was increased by adding three percent of ammonia to the sample solution.

Coupling of semi-micro LC and MS was achieved by increasing the gas-flow rate used to spray the solution to 6 L/min to reduce the density of solvent molecules in the spray. Using this method, a mixture of pesticides, i.e. simazine, thiuram, and thiobencarb, whose concentrations in drinking water are regulated in Japan, were simultaneously analyzed by semi-micro LC/MS. Although thiuram, which is especially thermolabile, generally has to be avoided when simultaneously analyzing other pesticides, it could be analyzed together with others when using SSI.

Coupling with a conventional LC was achieved by setting a multi-hole plate in front of the sampling orifice of the mass spectrometer to reduce the density of solvent molecules in the spray. Using this method, for the first time multiply-charged ions whose charge distribution ranged from 11+ to 16+ could be analyzed from a 1-μM cytochrome-c solution at a high solution-flow rate of 1 mL/min without using a splitter.

Coupling of CE and MS was achieved by adding a buffer reservoir between the sample-introduction capillary of the SSI interface and the electrophoresis capillary to

prevent the solution in the electrophoresis capillary being pumped by the pressure difference between the two ends of the capillary. Using this method, the author has demonstrated CE/MS analysis with a mobile-phase buffer containing 15 mM of phosphate, whose use has been generally avoided in CE/MS, by filling the buffer reservoir with an acetic-acid solution as a substitute for the mobile-phase buffer. This increased the ion intensity one-hundred fold by enhancing the evaporation of the charged droplets.

As described above, since SSI can be used to analyze many kinds of organic compounds, it enables the simultaneous analysis of complex mixtures. Also, SSI can be used with virtually any solution, unlike other interface techniques; for example, ESI and IS can only be used under a limited range of buffer conditions that depend on the solution conductivity. Thus, SSI enables coupling of a liquid separation tool and a mass spectrometer without lowering the separation ability of the liquid separation tool. Therefore, LC/MS and CE/MS using SSI are valuable analytical tools for liquid samples. These techniques are especially useful for the analysis of mixtures containing many contaminants. The author believes the SSI technique can make a valuable contribution to the evolution of various fields of science; for example, medical, environmental, and brain science. In addition, some techniques developed in this research have been incorporated in Hitachi's M-8000 liquid chromatograph / three-dimensional quadrupole mass spectrometer, which was commercially launched in August, 1997.

学術論文及び口頭発表目録

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