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A Sonic Spray Interface for Capillary Electrophoresis/Mass Spectrometry

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We have developed a sonic spray ionization interface that enables coupling of capillary electrophoresis (CE) and mass spectrometry (MS). In sonic spray ionization, a sample solution is sprayed at any solution-flow rate from a sample-introduction capillary with a high-speed gas flow coaxial to the capillary, and ions are formed at atmospheric pressure. Therefore, it is 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 expected to be decreased by the pumping effect when an electrophoresis capillary is connected directly to the sample-introduction capillary. To avoid this in our CE/MS interface, we added a buffer reservoir between the sample-introduction capillary of the ion source and the electrophoresis capillary. We confirmed that this prevents the solution in the electrophoresis capillary being pumped by the pressure difference. Furthermore, we have demonstrated analysis by CE/MS 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 100-fold by enhancing the evaporation of charged droplets produced by the spray.

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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 (UV) absorption is limited because the migration time is liable to significant shifts, 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] pneumatically assisted ESI,[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 for the delivery of a make-up flow in coupling by pneumatically assisted ESI, and also in the CF-FAB interface technique.[8] However, analysis by CE/MS has to be performed using a limited selection of mobile-phase buffers, because the use of nonvolatile buffers is generally avoided to protect the ESI interface. Although phosphates are widely used as a mobile-phase buffer for CE, their usable concentrations in CE/MS are restricted because sprays of high-conductivity solutions such as phosphate buffers are unstable in ESI.

Recently, we have developed a sonic spray ionization (SSI) interface for liquid chromatography (LC)/MS.[9–14] In this technique, a sample solution is sprayed from a sample-introduction capillary by a high-speed nitrogen gas flow that is coaxial to the capillary, and ions of chemicals in the solution as well as charged droplets are produced at room temperature and atmospheric pressure. Furthermore, an electric field is applied to the solution in the capillary to increase the non-uniformity of ion concentration at the solution surface in the capillary. This increases the charge density of the droplets, so that the ion intensity is enhanced and multiply-charged ions are produced.[11,14] Note that the electrical potential of the solution in the capillary is held at ground potential in this case; an electrospray aerosol is not generated and fine, charged droplets are produced from the droplets only by the shear stress due to the high-speed gas flow. Since it is not necessary to apply heat to the ion source to produce ions, thermolabile compounds such as peptides and neurotransmitters are readily analyzed.[9,10] The SSI interface that we developed was for use in semi-micro and conventional LC/MS.[12,13] Sprays produced using SSI are stable at any solution-flow rate and any buffer conditions, because sprays are generated only by gas flow. However the pressure around the tip of the sample-introduction capillary is reduced by a 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. Therefore, the resolution of CE separation is expected to be decreased in this case, even though ions are readily formed at any solution-flow rate. To prevent the solution in the electrophoresis capillary being pumped, we have developed a SSI interface specifically for CE/MS.

ESTIMATION OF PUMPING RATES

We began by estimating pumping rates into the capillary

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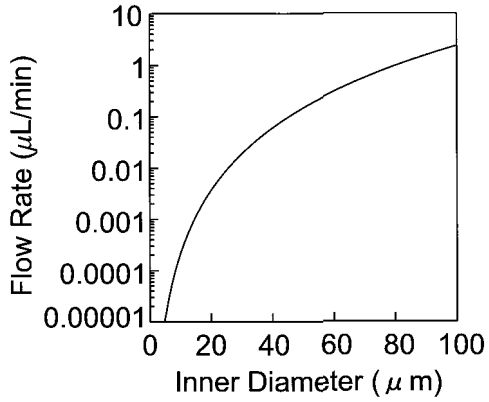

Figure 1. Dependence of the pumping rate on the i.d. of a 60-cm-long capillary.

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:

$$Q = \Delta p \pi r^4 / (8\mu L) \quad (1)$$

In Eqn. 1, Δp, r, μ and L, respectively, are the pressure difference between the two ends of the capillary, the capillary radius, the solution viscosity, and 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. We 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 is approximately 2×10^{-3} Pa/s;[15] accordingly Δp was approx. 0.2×10^5 Pa. Using this value, we estimated the dependence of the pumping rate on the internal diameter (i.d.) of a 60-cm-long capillary (Fig. 1). The pumping rate decreased with decreasing i.d., and the pumping rates of capillaries with an i.d. 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 i.d. of over 50 μm are most practical because it is difficult to inject a sample into a capillary with a smaller i.d. Therefore, we used a 50-μm i.d. capillary, in which the pumping rate is significant.

EXPERIMENTAL

Figure 2 is a cross-sectional view of the SSI ion source for CE/MS. A fused-silica capillary (the sample-introduction capillary; 50-μm i.d., 150-μm o.d., 10 cm long, GL Science, Tokyo, Japan) is 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 housing, since the fused-silica capillary is flexible. One end of the sample-introduction capillary is inserted into a duralumin orifice (0.28-mm i.d.) in the housing. The capillary tip extends 0.2 mm beyond the orifice. The center axes of the sample-introduction capillary and the orifice are aligned. Pressurized nitrogen gas flows into the housing, causing a gas flow through the orifice to the atmosphere. The flow rate of the nitrogen gas at the standard state of temperature and pressure, as determined

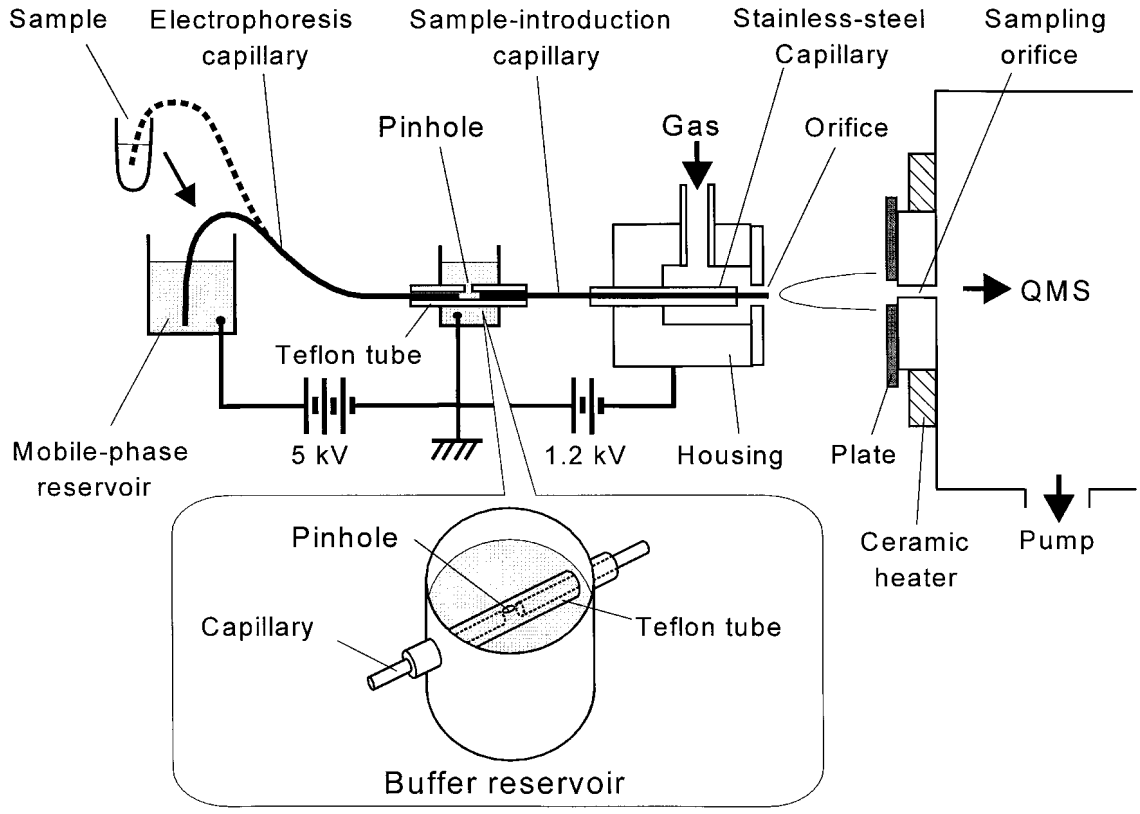

Figure 2. Cross-sectional view of the sonic spray interface for CE/MS, with a close-up of the buffer reservoir.

using a mass-flow controller (5850E, Brooks Instruments, Tokyo, Japan), is 2 L/min. A spray is thereby generated in which charged droplets and free ions are produced at atmospheric pressure. A voltage of −1.2 kV is applied to the orifice and the housing, which are isolated from the solution by the sample-introduction capillary, to increase the charge density of droplets produced by the gas flow.[11] The electrical potential of the solution in the sample-introduction capillary and a buffer reservoir is held at ground potential through a platinum electrode of the buffer reservoir. The ions produced in the atmosphere are introduced into the vacuum region of a quadrupole mass spectrometer through a sampling orifice (0.25-mm i.d., 25 mm long). The sampling orifice is heated with a ceramic heater to 120–130 °C and covered with a stainless-steel plate with a 2-mm aperture to avoid cooling of the sampling orifice due to the gas flow. The distance between the sample-introduction capillary tip and the surface of the plate is 3 mm. The details of the mass spectrometer have been described elsewhere.[12,16]

The other end of the sample-introduction capillary is inserted into a Teflon tube (0.15-mm i.d., 1.4-mm o.d.) that passes through the buffer reservoir (Fig. 2). The buffer reservoir is filled with a buffer solution so that the pinhole (whose diameter is about 0.1 mm) in the Teflon tube is submerged and the buffer solution flows through the pinhole into the sample-introduction capillary. A fused-silica capillary for electrophoresis (the electrophoresis capillary, 50-μm i.d., 150-μm o.d., GL Science, Tokyo, Japan) is inserted into the Teflon tube from the opposite end. The capillaries are set opposite to each other near the pinhole in the tube. The other end of the electrophoresis capillary goes into a mobile-phase reservoir filled with the buffer solution. A high voltage is applied between the buffer reservoir and the mobile-phase reservoir to generate electrophoresis. The sample solution separated by electrophoresis is mixed with the buffer solution in the area beneath the pinhole in the tube, and pumped into the sample-introduction capillary. Since the pumping rate of the 10-cm-long capillary is about 1 μL/min, as estimated from Eqn. 1, the solution from the buffer reservoir is 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 will not diffuse significantly into the buffer reservoir. The sample solution is 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 diameter of the sample-introduction capillary can differ from that of the electrophoresis capillary.

RESULTS AND DISCUSSION

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 were not observed. Therefore, the solution in the electrophoresis capillary was not pumped by the gas flow. This suggests that this interface could be used for CE/MS without any loss of resolution due to the pumping effect.

Figure 3 is an electrophorogram 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. We monitored the ion intensity at *m/z* 104 and 154. After 13 and 18 minutes, respectively, peaks corresponding to the dopamine and GABA ions were observed in the electrophorogram. The GABA and dopamine were clearly separated and detected. Separation efficiencies for the GABA and dopamine were about 10 000 and 30 000 theoretical plates, respectively.

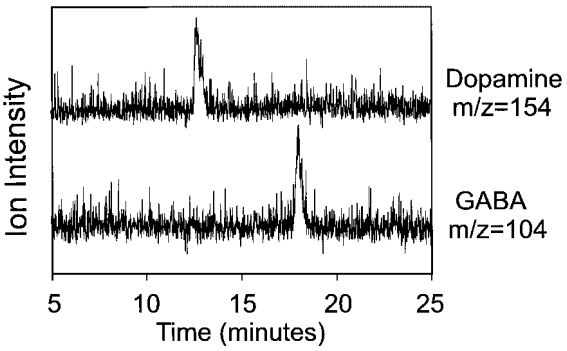

Figure 3. Electrophorogram of dopamine and GABA. Both reservoirs were filled with a 15 mM ammonium acetate buffer (pH 6.3) in water/methanol (50:50, v/v), and the injected samples were 12 and 15 pmol of dopamine and GABA, respectively. The electrophoresis capillary was 40-cm long and 5 kV was applied between the two reservoirs.

CE/MS using a phosphate buffer

With the SSI, solutions of any kind can be sprayed steadily because the solutions are sprayed only by the high-speed gas

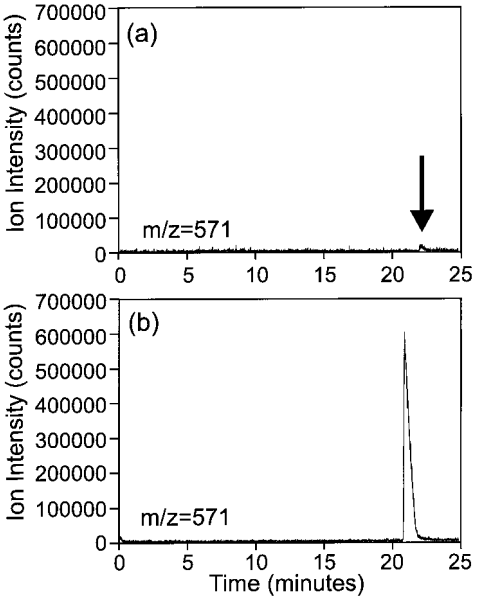

Figure 4. Electrophorograms of gramicidin-S. The buffer reservoir and the mobile-phase reservoir were filled with (a) a 15 mM phosphate buffer (pH 6.3) in water/methanol (50:50, v/v), or (b) a 15 mM phosphate buffer and an acetic-acid solution (42:50:8, water/methanol/acetic acid, pH 1.4). The injected sample was 2 pmol. The electrophoresis capillary was 40 cm long and 5 kV was applied between the two reservoirs.

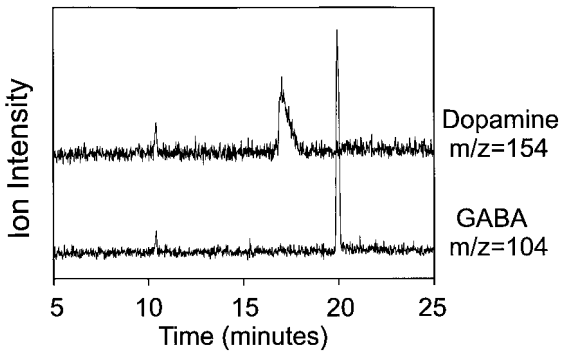

Figure 5. Electrophorogram of dopamine and GABA. The buffer reservoir and the mobile-phase reservoir were filled with a 15 mM phosphate buffer (pH 6.3) in water/methanol (50:50, v/v) and an acetic-acid solution (42:50:8, water/methanol/acetic acid, pH 1.4). The injected samples were 12 and 15 pmol of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

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 acid increases the ion intensity. Figure 4 shows electrophorograms of gramicidin-S, where 2 pmol of gramicidin-S were 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. 4(a)), or a 15 mM phosphate buffer and an acetic-acid solution (42:50:8, water/methanol/acetic acid, pH 1.4) (Fig. 4(b)). The signal/noise ratio of the gramicidin-S peak in Fig. 4(b) is about one-hundred times as high as in Fig. 4(a) because of the accelerated solvent evaporation due to the volatile substance added to the solution. Although a volatile buffer is also 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 above 15 mM can be used in this interface.

Figure 5 is an electrophorogram of the dopamine and GABA mixture. In this case, the mobile-phase reservoir was filled with a 15 mM phosphate buffer, and the buffer reservoir was filled with acetic acid. We injected 12 pmol of dopamine and 15 pmol of GABA and 5 kV was applied between the two reservoirs. We 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/noise ratios of the GABA and dopamine peaks in Fig. 5 were about 2 to 4 times higher than those in Fig. 3. Also, the peak width for GABA was much smaller than that for dopamine in Fig. 5. 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 analysis by CE/MS with virtually any condition 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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