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Evaluation of an atmospheric pressure chemical ionization interface for capillary electrophoresis–mass spectrometry

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Dedicated to Professor Terumichi Nakagawa on the occasion of his retirement and 63rd birthday.

Abstract

A simple and inexpensive approach to modify a commercial atmospheric pressure chemical ionization (APCI) interface was proposed for capillary electrophoresis–mass spectrometry (CE–MS). In order to accommodate lower flow rates in the range 1–10 μl/min, both sheath liquid and nebulizing gas were coaxially supplied to the nebulizer as an arrangement of pneumatically assisted electrospray interface. Since this paper focused on the primary study of the modified APCI interface, the performance of the interface was first evaluated by the direct infusion of a reserpine solution. Optimization of several APCI parameters, such as temperature of APCI vaporizer, nebulizing gas flow and APCI corona discharge current, were accomplished. The orifice dimension for the nebulizing gas flow was largely independent of the MS sensitivity when the nebulizing gas flow rate was ca. 0.4 l/min. A successful CE–APCI–MS separation is obtained using the modified APCI interface.

Keywords: Capillary electrophoresis–mass spectrometry; Modified interface; Atmospheric pressure chemical ionization

1. Introduction

Mass spectrometry (MS) is one of the powerful detectors for analytical separation techniques because of its high sensitivity and capability to identify chemical compounds. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are widely used as very soft ionization techniques particularly in liquid chromatography–mass spectrometry (LC–MS) [1,2]. Because standard ESI sources for LC–MS can also be handled at flow rates lower than 10 μl/min, they are compatible with a direct coupling of capillary electrophoresis (CE) and MS using sheath liquid flow without difficulties [3]. Hence, a number of CE–ESI–MS applications have been

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published over the last decade [3,4]. On the contrary, standard APCI sources accommodate high flow rates in the range 0.1–1.5 ml/min [1]. Although APCI is expected to give better sensitivity than ESI for less polar compounds, current commercial APCI interfaces are inadequate for on-line CE–MS since a large sheath liquid flow dilutes the sample excessively, resulting in a loss of signal. To date, a few laboratory-built or custom-made APCI interfaces have been developed for open tubular LC–APCI–MS [5] and CE–APCI–MS [6–8] to accommodate liquid flow rates lower than 10 μl/min. In our previous papers [7,8], a custom-made APCI interface having an arrangement of electrospray process prior to chemical ionization was used for CE–APCI–MS. A mixture of CE solution and sheath liquid were nebulized into the APCI vaporizer with the aid of electrospray. However, good concentration sensitivity could not be obtained probably due to the low-efficiency sample introduction to the APCI vaporizer. In this paper, a simple and inexpensive approach to modify a commercial APCI interface is introduced. It is accomplished by coaxial additions of sheath liquid flow and nebulizing gas in the nebulization process. The fabrication, properties and application of the modified APCI interface are described.

2. Experimental

2.1. Apparatus

An LCQ ion-trap mass spectrometer (Thermo Quest, Tokyo, Japan) equipped with a standard APCI interface was used. The APCI interface was modified to accommodate a coaxial addition of sheath liquid flow using a stainless steel tube (27G or 26G) and a tee union (Valco International, Schenkon, Switzerland) as shown in Fig. 1. One end of the stainless steel tubes was tapered with sandpaper as given in Table 1. The screw hole on the APCI manifold was enlarged with a drill for the stainless steel tube to penetrate. For direct infusion experiments, a fused-silica capillary of 50 μm i.d. and 150 μm o.d. (Polymicro Technologies, Phoenix, AZ) was used. For CE–MS, a fused-silica capillary of 60 cm total length, 50 μm i.d. and 186 μm o.d. (Polymicro Technologies) was used. A high voltage of 15 kV was applied to the inlet electrode using a DC power supply (model HCZE-30PN0.25-LDSW, Matsusada Precision, Shiga, Japan).

2.2. Reagent

Reserpine and pindolol were purchased from Wako (Tokyo, Japan); nicardipine and trimipramine were purchased from Sigma (St. Louis, MO); and sulpiride was from Research Biochemical (Natick, MA). Water was purified with a Milli-Q Labo system (Nihon Millipore, Tokyo, Japan). All other reagents were of analytical or HPLC grade.

2.3. Procedure

Nitrogen was used as a nebulizing gas (ca. 0.4 l/min) and an auxiliary gas (negligible in comparison with the nebulizing gas flow). APCI vaporizer was heated to 350 °C to provide optimum desolvation. APCI corona discharge current was set at 2 μA. Prior to direct infusion experiments and CE–MS analyses, a sample solution such as 1 μg/ml reserpine solution was infused at 5 μl/min using a syringe pump equipped for an LCQ instrument. In incorporating a fused-silica capillary and a stainless steel tube into the APCI nozzle, the positions of their tips should be carefully adjusted to obtain the highest peak intensity of the protonated molecular ion of the sample. Subsequently, an automatic tuning was performed to establish the optimized MS parameters. For direct infusion experiments, reserpine was dissolved in a mixture of 50 mM ammonium formate and methanol (1:1) at the concentration of ca. 1 μg/ml. The reserpine solution was infused at 5 μl/min unless otherwise mentioned, and the data acquisitions were performed using full scan mode (300–800 m/z). For CE–MS separations, a 50 mM ammonium formate buffer (pH 5.0) was used as a separation buffer. A sample solution was prepared in a mixture of water and methanol at the concentration of approximately 25–50 μg/ml. As a sheath liquid, a mixture of the separation buffer and methanol (1:1) was supplied at 10 μl/min using a

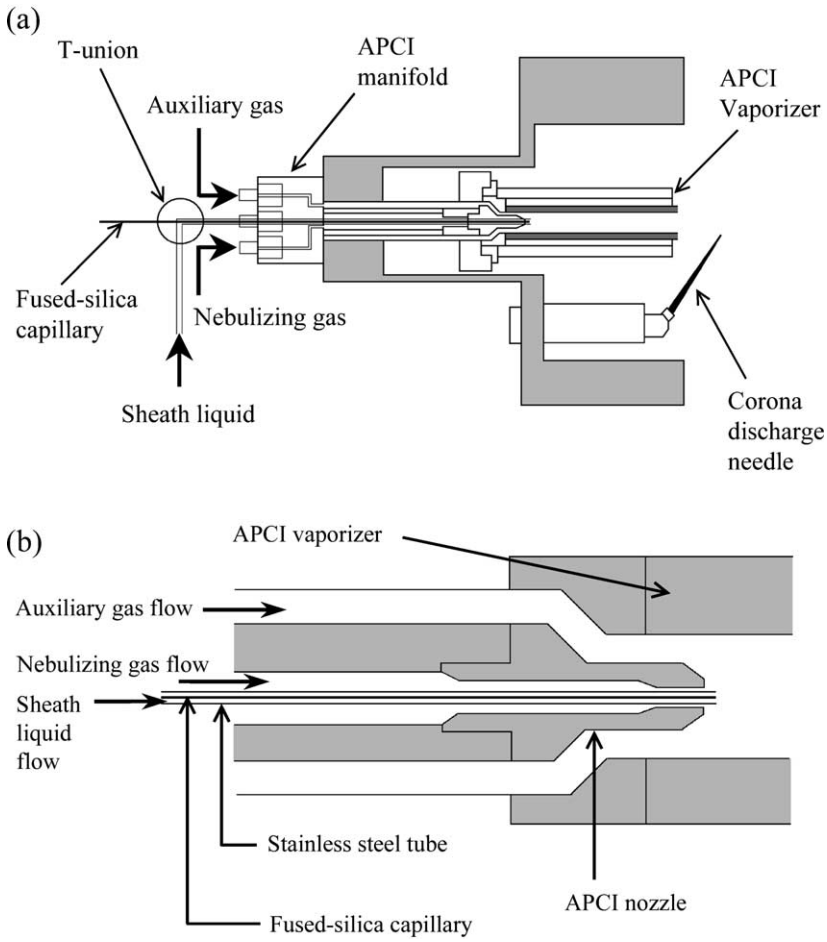

Fig. 1. Schematic diagram of (a) the modified APCI interface for CE–MS and (b) details of coaxial sheath-flow configurations in the APCI interface.

Harvard apparatus syringe pump (Model 11, South Natick, MA). All solutions were filtered through a 0.22 μm syringe-type membrane filter prior to use. A CE capillary was conditioned for its first use by rinsing it with ca. 20 column volumes of 0.1 M NaOH followed by ca. 20 column volumes of water and more than 10 column volumes of the electrolyte buffer. A sample solution was injected hydrodynamically at 10 cm height for 10 s, the injection end of the capillary was dipped into the electrolyte buffer, and a constant voltage of 15 kV was applied. Data acquisitions were performed using selected ion monitoring mode at each protonated molecular

Table 1
Stainless steel tube dimensions used for the evaluation of the modified APCI interface

Gage	Outer diameter (μm)	Inner diameter (μm)	Tapered outer diameter at the tip toward the APCI nozzle (μm)
27G	410	190	320, 340, 360, 380, 400
26G	450	230	400

mass of analytes. After the capillary was rinsed with the separation buffer, the next analysis was performed.

3. Results and discussion

3.1. Fabrication of the APCI interface

When 1 μg/ml reserpine solution was directly infused at 50 μl/min into an APCI interface equipped for an LCQ instrument without modifications, the protonated molecular ion peak of reserpine was not observed. In order to accommodate lower flow rates in the range 1–10 μl/min, the modification of the APCI interface was attempted. Fig. 1 shows schematic illustration of the modified APCI interface, which is a similar design as a pneumatically assisted ESI interface. It allowed stable nebulization, desolvation of liquids in the APCI vaporizer and transfer of the ions from the capillary into the MS detector at the lower flow rates. As shown in Fig. 2, the successful MS spectra of reserpine were obtained by direct infusion experiments. Since liquid flow from CE capillary driven by the electroosmotic force is very little, a reserpine solution of ca. 100 μg/ml was infused at 0.5 μl/ml with a sheath liquid at 5 μl/min. In this experiment, stable MS spectrum of reserpine was also successfully obtained as in Fig. 2 (data not shown).

3.2. Evaluation of the APCI interface by direct infusion of reserpine solution

3.2.1. Temperature of the APCI vaporizer

Before beginning CE–MS separations, the modified APCI interface was evaluated for the optimum conditions by infusing a reserpine solution. A high temperature is applied to the APCI interface to volatilize analytes for gas-phase ion/molecule reactions with primary ions that are generated from a reagent gas by a corona discharge. As shown in Fig. 3, the vaporization of the sample solution was complete at 275 °C and above, and the changes in the APCI vaporizer temperature did not significantly affect the sensitivity. In our experiments, therefore, the temperature was set

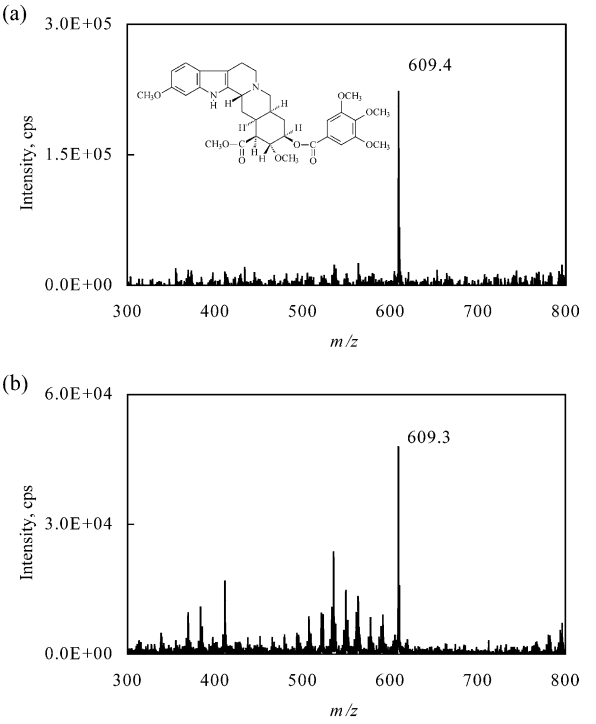

Fig. 2. APCI–MS spectra of reserpine by direct infusion experiments. Sample solution, reserpine of (a) 1 μg/ml and (b) 0.1 μg/ml dissolved in a mixture of 50 mM ammonium formate and methanol (1:1); flow rate of sample infusion, 5 μl/min; temperature of APCI vaporizer, 350 °C; flow rate of nebulizing gas, 0.4 l/min; corona discharge current, 2 μA.

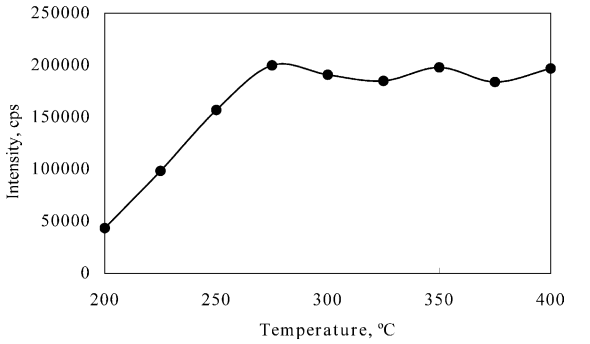

Fig. 3. Dependence of peak intensity of reserpine versus APCI vaporizer temperature. Sample infusion, 1 μg/ml reserpine solution at 5 μl/min. Other conditions are as in Fig. 2.

at 350 °C. Because most of the heat is used in evaporating the sheath liquid and heating the neburizing gas, the thermal effect on the sample

is much less expected. However, too high a temperature causes thermal degradation of the molecular ion for very thermally labile compounds and thus decreases sensitivity. This parameter must be optimized if the thermal degradation of analytes is observed.

3.2.2. Nebulizing gas flow rate

The signal intensity of reserpine was dependent on various APCI parameters. The effects of nebulizing gas flow rate were studied in the range 0.2–1.5 l/min. Although the optimum gas flow rate slightly depended on the sample infusion rate as shown in Fig. 4, the most intensive signal was obtained at approximately 0.4 l/min. The signal intensity was linearly increased as a function of the sample infusion rate in the range 1–20 μl/min (data not shown). It suggested that the APCI interface showed good concentration sensitivity in accordance with the total sample amounts infused into the APCI vaporizer.

3.2.3. APCI corona discharge current

Since a corona discharge is used to ionize the reagent gas in the atmospheric pressure region, the corona discharge current is one of the APCI parameters. We varied the corona discharge current in the range 0.5–8 μA. As shown in Fig. 5, however, no significant effect of the peak intensity was observed. The corona discharge current was set at 2 μA in our experiments. If it requires maximum sensitivity, optimization of the APCI corona discharge current should be necessary.

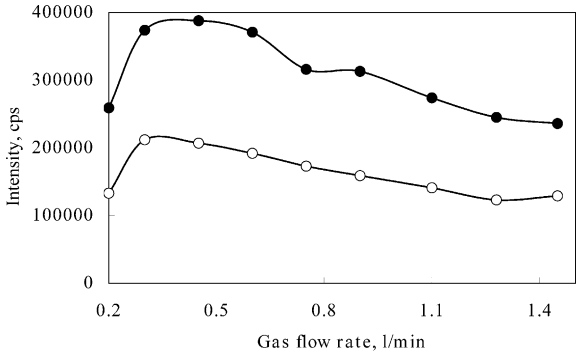

Fig. 4. Effects of nebulizing gas flow and sample infusion rate on the peak intensity of reserpine. Sample infusion, 1 μg/ml reserpine solution at 5 μl/min (open circle) and 10 μl/min (closed circle). Other conditions are as in Fig. 2.

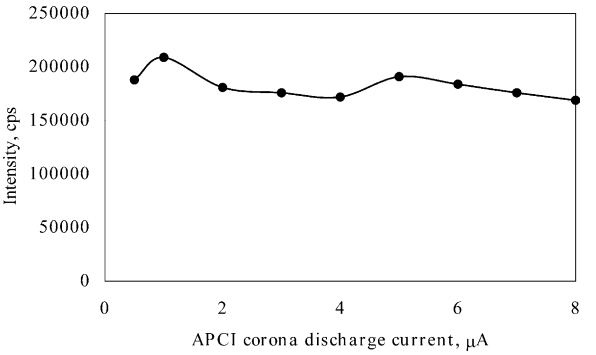

Fig. 5. Dependence of peak intensity of reserpine versus APCI corona discharge current. Conditions are as in Fig. 3.

3.2.4. Tip diameter of stainless steel tube at the APCI nozzle

As for ESI interface, it was previously reported that the dimensions of the three concentric outlets, i.e. CE solution, sheath liquid flow and nebulizing gas, showed to affect the performance of the system in terms of both sensitivity and stability [9]. Therefore, the effects of the different orifice dimension for the nebulizing gas flow were evaluated using the modified APCI interface. Since the APCI nozzle used in this study has an internal diameter of approximately 410 μm, one end of the stainless steel tube must be tapered to penetrate the nozzle. Several stainless steel tubes having different tapered diameters as listed in Table 1 were prepared. The optimum nebulizing gas flow rate was ca. 0.4 l/min to obtain the maximum MS sensitivity independent of other operating parameters. That is to say, the sheath gas parameter (in arbitrary units) on the LCQ instrument had to be increased to reach the gas flow rate of 0.4 l/min as the tapered outer diameter of the stainless steel tube increased due to an enhanced restriction for the neburizing gas flow. Unlike the ESI interface, however, the orifice dimension was largely independent on the sensitivity and stability (data not shown). It affected the performance of sample nebulization only, which was optimized by adjusting the neburizing gas flow rate, but not the ionization.

3.3. CE–APCI–MS separation

In CE–MS, a narrow capillary of 50 μm i.d. and 150 μm o.d. tended to break in use caused by the high electric field and by the heat generation. Based on our experience [10], we employed a fused-silica capillary of 186 μm o.d. and a 26G stainless steel tube in CE–APCI–MS. A low concentration of ammonium formate buffer was chosen for the electrophoretic buffer because MS detection required volatile electrolytes. A sheath liquid was supplied at 5 μl/min in our preliminary experiments, but unstable MS signals were occasionally observed presumably due to the breaking electrical contact across the sheath liquid. This problem was solved by increasing the sheath liquid flow rate up to 10 μl/min. Fig. 6 shows selected ion electropherograms of test samples obtained by CE–APCI–MS. In this study, some representative polar compounds were used to perform a fundamental approach of CE–APCI–MS, even if these analytes can be readily ionized by ESI. In addition, further optimization of the operating conditions was not attempted. The actual performance may differ considerably by variations in experimental parameters and samples. For less polar compounds, a better sensitivity would be obtained than ESI.

4. Conclusion

ESI and APCI are complementary methods as soft ionization techniques to cover a wider range of samples. It may be difficult to decide which is more appropriate technique as definitive rules. A general observation is that APCI tends to give better sensitivity than ESI for less polar compounds. Nevertheless, CE–APCI–MS has not been practically used as compared with LC–MS because commercial APCI interfaces accommodate high flow rates in the range 0.1–1.5 ml/min. In this paper, we described a modified APCI interface to accommodate flow rates lower than 10 μl/min including sheath designs for implementation of CE–APCI–MS. It has an advantage that switching between ESI and APCI takes minutes without difficulties as well as LC–MS. Recently, a

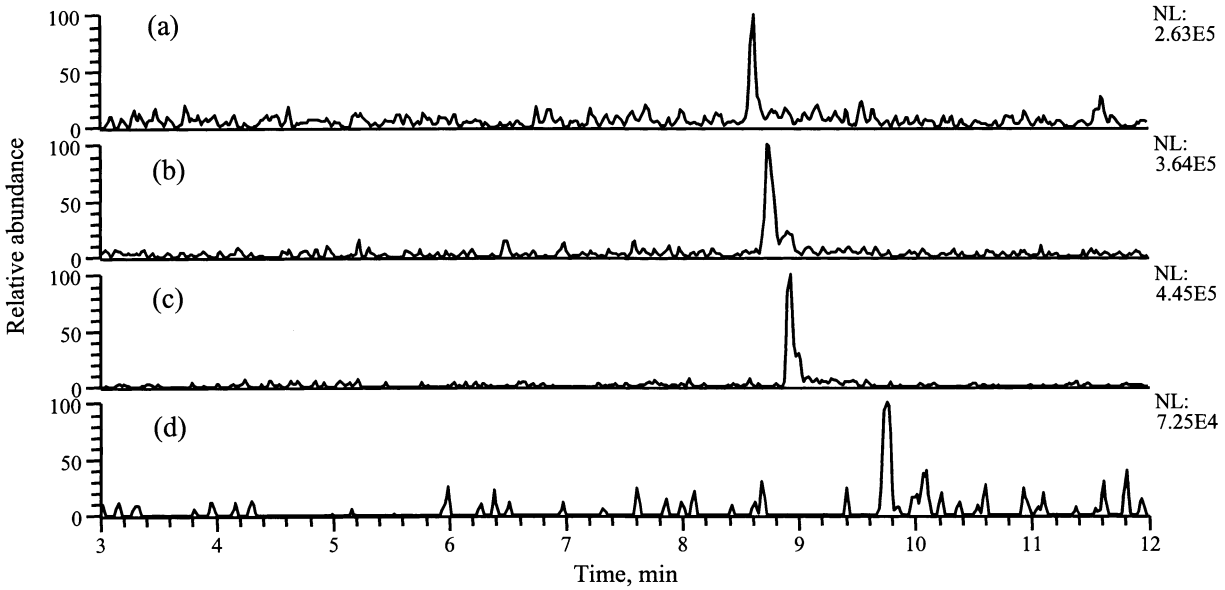

Fig. 6. Selected ion electropherograms of (a) pindolol, (b) trimipramine, (c) sulpiride and (d) nicardipine obtained by CE–APCI–MS. CE buffer, 50 mM ammonium formate buffer (pH 5.0); sheath liquid, a mixture of the CE buffer and methanol (1:1); sample concentration, (a–c) 50 ppm and (d) 25 ppm in a mixture of water and methanol; CE voltage, 15 kV; CE current, ca. 32 μA; MS detection (m/z), (a) 248.7–249.7, (b) 294.7–295.7, (c) 341.7–342.7 and (d) 479.8–480.8.

miniaturization of analytical separation instruments has progressed. The modified APCI interface can be readily applicable to the coupling of MS and micro-scale separation techniques including micro-HPLC as well as CE.

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