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Separation of enantiomers by capillary electrophoresis–mass spectrometry employing a partial filling technique with a chiral crown ether

Yoshihide Tanaka[a,*], Koji Otsuka[b], Shigeru Terabe[b]

[a]*Department of Analytical Chemistry, Nippon Boehringer Ingelheim Co. Ltd., 3-10-1, Yato, Kawanishi, Hyogo 666-0193, Japan*
[b]*Faculty of Science, Himeji Institute of Technology, 3-2-1, Kouto, Kamigori, Hyogo 678-1297, Japan*

Abstract

Enantiomer separations were performed by capillary electrophoresis–mass spectrometry (CE–MS) with (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid ($18C6H_4$) as a chiral selector. In order to prevent the introduction of the nonvolatile chiral selector, $18C6H_4$, into the nozzle of the CE–MS interface and/or the orifice plate, a partial filling technique was employed in this study. By the partial filling technique, the contamination caused by the nonvolatile chiral selector was avoided not only during the analysis but also during the washing of capillary with the separation solution prior to the run. Several racemic compounds having a primary amino group were successfully separated. Racemic 3-aminopyrrolidine and racemic α-amino-ϵ-caprolactam have no strong UV absorption, but such compounds were detected with a high sensitivity by MS detection. In this paper, the effects of the length of separation zone and those of the $18C6H_4$ concentration were described. As the length of the separation zone was longer or as the concentration of $18C6H_4$ was higher, the enantiomer resolution was enhanced more and more. However, the optimization of $18C6H_4$ concentration was practically enough to obtain the baseline separation.

Keywords: Enantiomer separation; Partial-filling capillary electrophoresis; Crown ethers; Aminopyrrolidine; Aminocaprolactam; Cycloserine

1. Introduction

Capillary electrophoresis (CE) has been found to be a powerful technique for enantiomer separations and a number of applications have been already described in some review articles, especially in pharmaceutical field [1–3]. Since most compounds have strong UV absorption, CE instruments equipped with an UV detector have been widely employed until now. However, coupling of CE with mass spectrometry (MS) detection offers several advantages over UV detection method. Analytes having no strong UV absorption are detected with a high sensitivity by MS detection. Even if some interfering compounds are co-eluted to MS detector, analytes are detected with a high selectivity according to the specific molecular mass. Furthermore, structural information of the analytes is obtained simultaneously on the separation. Recently, several researcher

*Corresponding author. Tel.: +81-727-90-2322; fax: +81-727-93-8399.

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groups reported on enantiomer separations by CE–MS using cyclodextrin (CD) derivatives [4–10], egg white avidin [8] and vancomycin [11] as chiral selectors. In CE–MS, nonvolatile components in the separation solution contaminate the nozzle of the CE–MS interface and/or the orifice plate. It may also cause a decrease in ionization efficiency of analytes at the interface. Either ammonium formate or ammonium acetate buffer is usually employed as a volatile electrolyte, but a nonvolatile chiral selector, such as CDs and proteins, must be added to the volatile electrolyte in order to obtain enantiomer separations. To avoid the introduction of the chiral selector into the interface, several procedures have been introduced. Lamoree et al. developed a heart-cut technique using a liquid junction system [6] and switching voltage from the separation capillary to the transfer capillary, but a specific interface is necessary to employ this technique. While, the problem is also solved on the basis of the opposite migration direction of an analyte to a chiral selector using a coated capillary where the electroosmotic flow is suppressed [11]. A partial filling technique is a kind of procedure [8,10], which is easily performed using a commercial interface without further modification. The partial filling technique has several advantages in comparison to the conventional procedure. When a capillary is washed with a separation solution prior to the run, the separation solution is blown into the orifice plate, and a nonvolatile chiral selector may contaminate it even if the electrospray voltage is interrupted during the washing of capillary. In the partial filling technique, however, the capillary is partially filled with a separation solution only. Furthermore, it is unlikely to introduce the chiral selector into the interface during the analysis even if the electroosmotic flow is slightly generated due to the deterioration of the coated capillary. Accordingly, electronically neutral chiral selectors as well as charged ones can be employed without specific operations by this technique.

In this paper, a chiral crown ether derivative, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid ($18C6H_4$), was used as a chiral selector. $18C6H_4$ is one of the most powerful chiral selectors, especially for the separation of compounds having a primary amino group [12]. Several primary amines were successfully separated by CE–MS employing the partial filling technique.

2. Experimental

2.1. Apparatus

A Hewlett-Packard CE 3D instrument (Yokogawa Analytical Systems, Tokyo, Japan) was employed for the CE separation. A Perkin–Elmer Sciex API-300 triple quadrupole MS instrument (Perkin–Elmer Japan, Yokohama, Japan) was employed as the detector. A Hewlett-Packard Vectra XM Series 3 (5/120) computer was used for the control of the CE instrument, and a Macintosh one (model 8500/120) was used for the control of the MS instrument and data collections. A pneumatically assisted electrospray (ionspray, ISP) interface supplied by Perkin–Elmer Sciex was employed for the coupling of CE and MS. A fused-silica capillary of 50 μm I.D. and 180 μm O.D. (Spelco, Bellefonte, PA, USA) was coated with a linear polyacrylamide [13]. The capillary of 80 cm in total length was incorporated into a user assembled capillary cartridge supplied by Hewlett-Packard. A Harvard Apparatus syringe pump (Model 11, South Natick, MA, USA) was used for the delivery of a sheath liquid.

2.2. Reagent

Racemic 3-aminopyrrolidine was purchased from Tokyo Kasei (Tokyo, Japan). Racemic α-amino-ε-caprolactam and racemic cycloserine were purchased from Sigma (St. Louis, MO, USA). (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid ($18C6H_4$) was purchased from Fluka (Buchs, Switzerland). Water was purified with a Milli-Q Labo system (Nihon Millipore, Yonezawa, Japan). All other reagents were of analytical or HPLC grade.

2.3. Procedure

For the optimization of MS conditions, a sample was dissolved in a mixture of 40 m*M* ammonium formate solution−methanol (1:1, v/v) at a concentration of ca. 50 μg/ml. The solution was infused into the ISP interface directly at 5 μl/min with a Harvard Apparatus syringe pump. The ISP voltage was maintained at 5 kV. MS conditions were optimized in order to produce the highest peak intensity of each quasi-molecular ion.

For enantiomer separations by CE–MS, 40 m*M*

ammonium formate buffer (pH 4.0) was used as a running buffer. A separation solution was prepared by dissolving 18C6H_4 as a chiral selector in the running buffer. A sample solution was prepared in a mixture of water and methanol at the concentration of ca. 50 μg/ml. A mixture of 40 m*M* ammonium formate buffer (pH 4.0)−methanol (1:1, v/v) was delivered at 5 μl/min as a sheath liquid. All solutions were filtered through a 0.22 μm syringe type membrane filter prior to use. A partial filling technique was employed as described in a previous paper [8]. Fig. 1 shows the principle of the partial filling technique and the procedure is briefly described as follows. At beginning and end of each day, a capillary was washed with the capillary wash solution (Bio-Rad, catalogue No. 148-5022) and water at 94 kPa (940 mbar) for more than 10 min each. The capillary was rinsed with a running buffer for 5 min at 94 kPa (940 mbar) prior to each run (Fig. 1a), and was filled with a separation solution at 5 kPa (50 mbar) for a given (Fig. 1b). The length of the separation solution was about 55 cm (ca. 70% of the total length) when it was injected for 15 min. A sample solution was injected at 5 kPa (50 mbar) for 8 s, the injection end of the capillary was dipped into the running buffer, and a constant voltage of 25 kV was applied. The ISP voltage was not applied during sample injection and for 1 min from each start of the run, and then 5 kV was applied at another end of the capillary (the net voltage across the capillary was 20 kV). The enantiomers, which migrated towards the MS detector, were separated while they are in the separation solution (Fig. 1c). After passing through the separation solution, both enantiomers migrated at the identical velocities in the running buffer (Fig. 1d). Accordingly, the chiral selector was not introduced into the interface not only during the analysis but also the construction of the separation zone in the capillary for the preparation of the analysis. The MS detection was performed in the selected ion monitoring (SIM) mode for the positive quasi-molecular ion. After the capillary was rinsed with the running buffer, the next analysis was performed continuously.

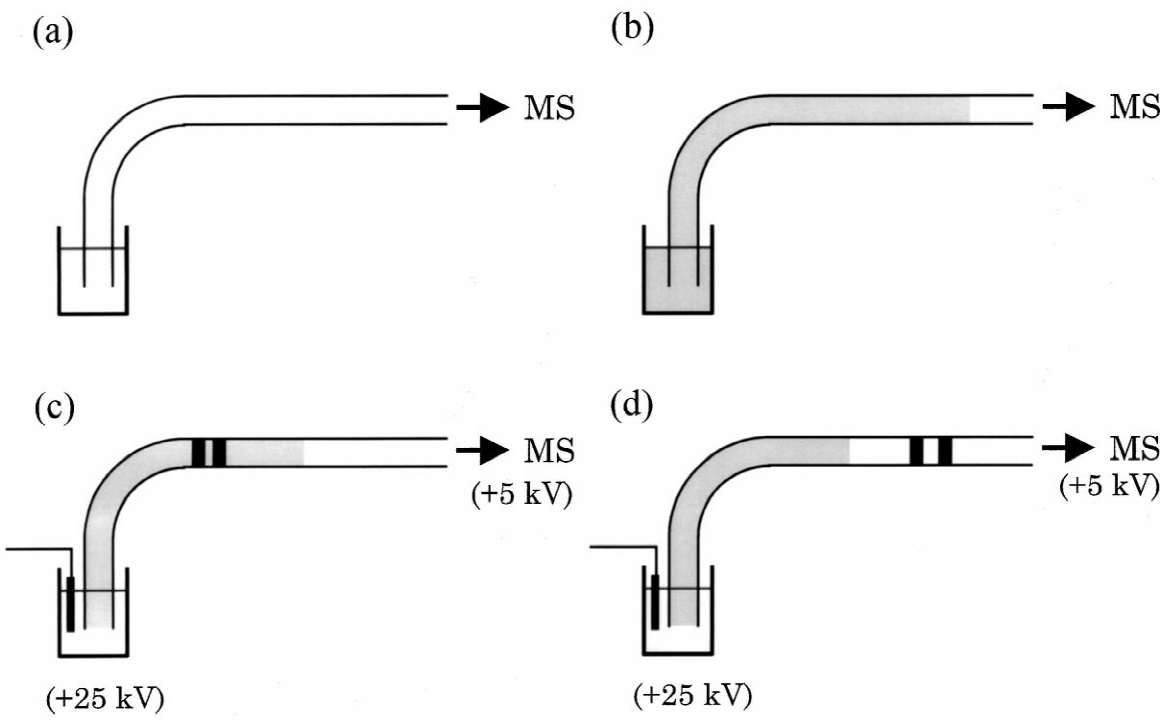

Fig. 1. Schematic illustration of the partial filling technique by CE–MS. White: running buffer, grey: separation solution, black: cationic analyte. Capillary, polyacrylamide-coated capillary; CE voltage, 25 kV; ISP voltage, 5 kV.

3. Results and discussion

3.1. Effects of the length of separation zone in the partial filling technique

When the partial filling technique is applied to enantiomer separations in CE–MS, the length of separation solution constructed in the capillary influences the enantiomer resolution because the enantiomer separations are achieved in the separation zone only. The length of separation zone was estimated to be about 55 cm (ca. 70% of total length) when it was injected for 15 min from a previous experiment with 50 m*M* heptakis(2,6-di-*O*-methyl)-β-CD [8]. Although it will be slightly different in the actual length due to the difference in viscosity of separation solutions containing $18C6H_4$ and the CD, the slight difference in the length is not important in this study. Therefore, the length of the separation solution was not determined using a $18C6H_4$ solution.

The effects of the length of separation zone were studied on the separation of racemic 3-aminopyrrolidine as an example. In order to optimize the MS conditions, racemic 3-aminopyrrolidine solution was infused into the ISP interface directly. As shown in Fig. 2, although several contaminant peaks caused by the solution were detected, the positive quasi-molecular ion of 3-aminopyrrolidine was obtained at *m*/*z* 87.0. In CE–MS, the selected ion monitoring mode was employed as the MS detection. Fig. 3 shows the effects of the length of separation zone by the partial filling technique. As the injection time of the separation solution is longer, namely, the length of the separation zone is longer; the enantiomer resolution is higher. Since the EOF was almost completely suppressed, the separation zone migrated towards the opposite direction of the MS detector during the analysis due to the electrophoretic mobility of anionic $18C6H_4$ itself as shown in Fig. 1. When the injection time was extended for more than 15 min, the enantiomer resolution should be increased. On the other hand, the enantiomer resolution was also enhanced by increasing the concentration of $18C6H_4$ as shown in Fig. 3d. Accordingly, the injection time was fixed at 15 min.

3.2. Effects of the concentration of chiral selector

The effects of $18C6H_4$ concentration on the separations of racemic 3-aminopyrrolidine were investigated by using 40 m*M* ammonium formate buffer (pH 4.0) within the range of 1–5 m*M*. Although enantiomer separations are achieved only in the partially filled separation solution, the effect of the concentration of the chiral selector can be discussed as in the conventional enantiomer separations, provided the separation zone length is kept constant. When the $18C6H_4$ concentration is increased, enantiomer resolution is enhanced initially

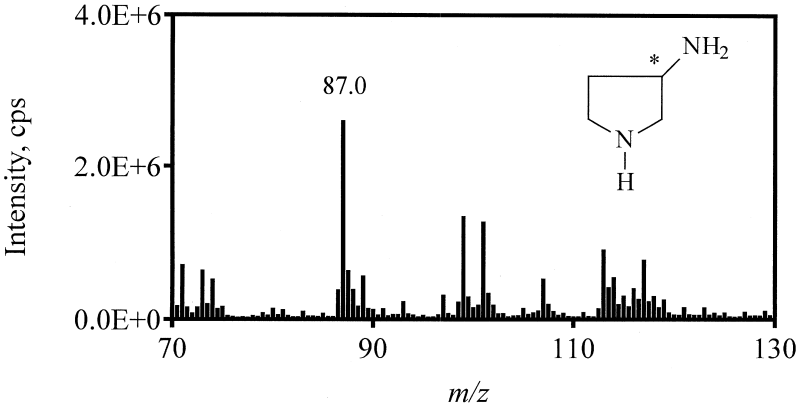

Fig. 2. MS spectrum of racemic 3-aminopyrrolidine on the optimization of MS conditions. Sample solution, racemic 3-aminopyrrolidine in a mixture of 40 m*M* ammonium formate solution–methanol (1:1, v/v), 50 μg/ml. Conditions: direct infusion at 5 μl/min with syringe pump; ISP voltage, 5 kV; MS detection, *m*/*z* 70 to 130 by 0.5 u (positive ion mode). Other MS conditions were optimized to obtain the highest peak intensity of the positive quasi-molecular ion (*m*/*z* 87.0).

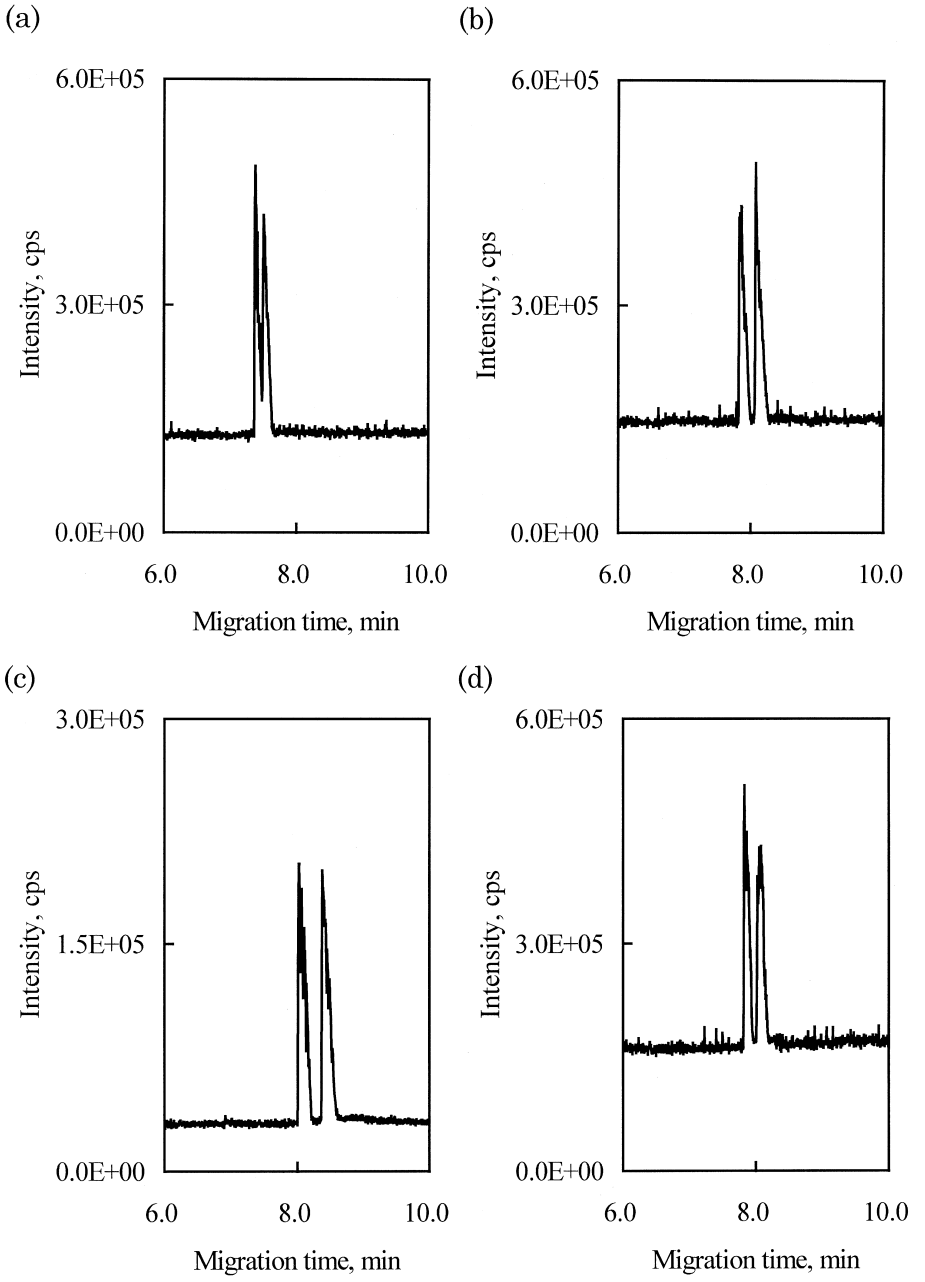

Fig. 3. Effects of the length of the separation zone on the separation of racemic 3-aminopyrrolidine. Conditions: capillary, 80 cm×50 μm I.D. linear polyacrylamide-coated capillary; sample solution, racemic 3-aminopyrrolidine in water and methanol, 50 μg/ml; sample injection, 5 kPa, 8 s; running buffer, 40 m*M* ammonium formate buffer (pH 4.0); separation solution, (a–c) 1 m*M*, (d) 2 m*M* 18C6H$_4$ in the running buffer; injection time of the separation solution, 5 kPa, (a, d) 5 min, (b) 10 min, (c) 15 min; CE voltage, 25 kV; ISP voltage, 5 kV; MS detection, m/z 87.1 (positive ion mode).

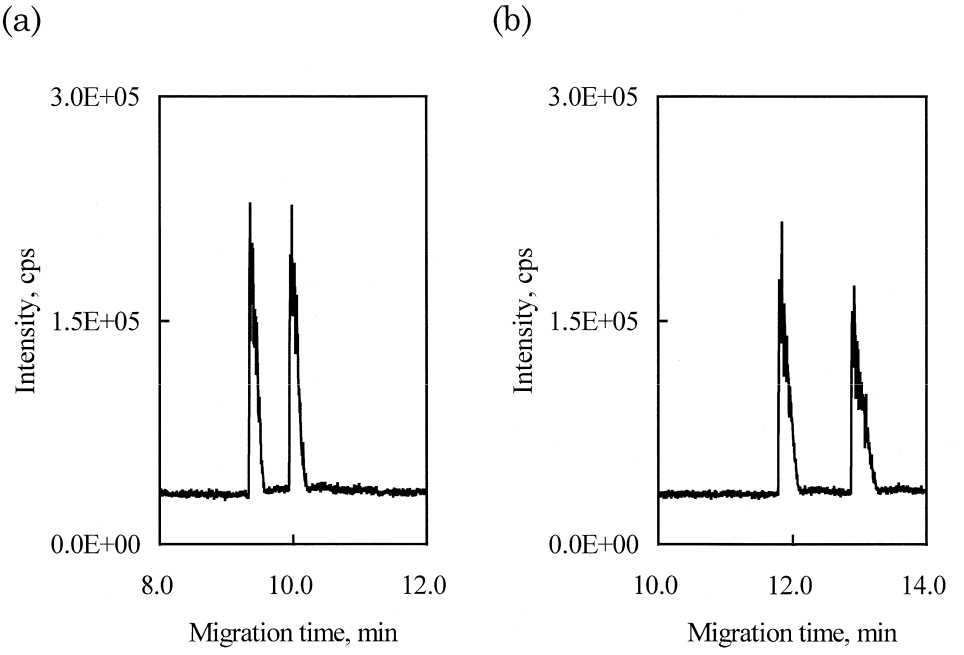

Fig. 4. Effects of the $18C6H_4$ concentration on the separation of racemic 3-aminopyrrolidine. Conditions: separation solution, (a) 2 m*M*, (b) 5 m*M* $18C6H_4$ in the running buffer; injection time of the separation solution, 5 kPa, 15 min. Samples and other conditions are as in Fig. 3.

but the apparent mobilities of the enantiomers are decreased. As shown in Figs. 3c and 4, the separation factor (the ratio of the migration times) was increased from 1.04 at 1 m*M* $18C6H_4$ to 1.09 at 5 m*M* $18C6H_4$. By further increasing the $18C6H_4$ concentration, the enantiomers should migrate toward the anode, which is the same as the migration direction of anionic $18C6H_4$. The enantiomer resolution should be improved by increasing the $18C6H_4$ concentration at more than 5 m*M*, but 1 m*M* was practically enough to obtain the baseline separation as shown in Fig. 3c.

3.3. Enantiomer separation of racemic compounds

Because 3-aminopyrrolidine has no strong UV absorption, it is difficult for such compound to detect with an UV detector. However, it can be detected with a high sensitivity by MS as shown in Figs. 3 and 4. Racemic α-amino-ϵ-caprolactam is also one of the compounds having a weak UV absorption and the enantiomer separation is shown in Fig. 5a. Thus, CE–MS is demonstrated to have an attractive feature for the detection of enantiomers having a weak UV absorption. Fig. 5b shows the separation of racemic cycloserine by CE–MS. This compound can be detected with an UV detector. However, the information of the molecular mass is simultaneously obtained by MS detection. The analyte should be detected according to the specific molecular mass even if some interfering compounds are co-eluted to MS detector.

4. Conclusion

Enantiomer separations were performed by CE–MS employing the partial filling technique. A merit of MS detection is that the analytes having a weak UV absorption can be detected with a high sensitivity. Several enantiomers including weak UV absorbing compounds were successfully separated with $18C6H_4$ as a chiral selector. $18C6H_4$ is a powerful chiral selector for the enantiomer containing a primary amino group, and a number of enantiomer separations by CE employing an UV detection method have been introduced in the review [12]. By changing the buffer electrolyte from a nonvolatile

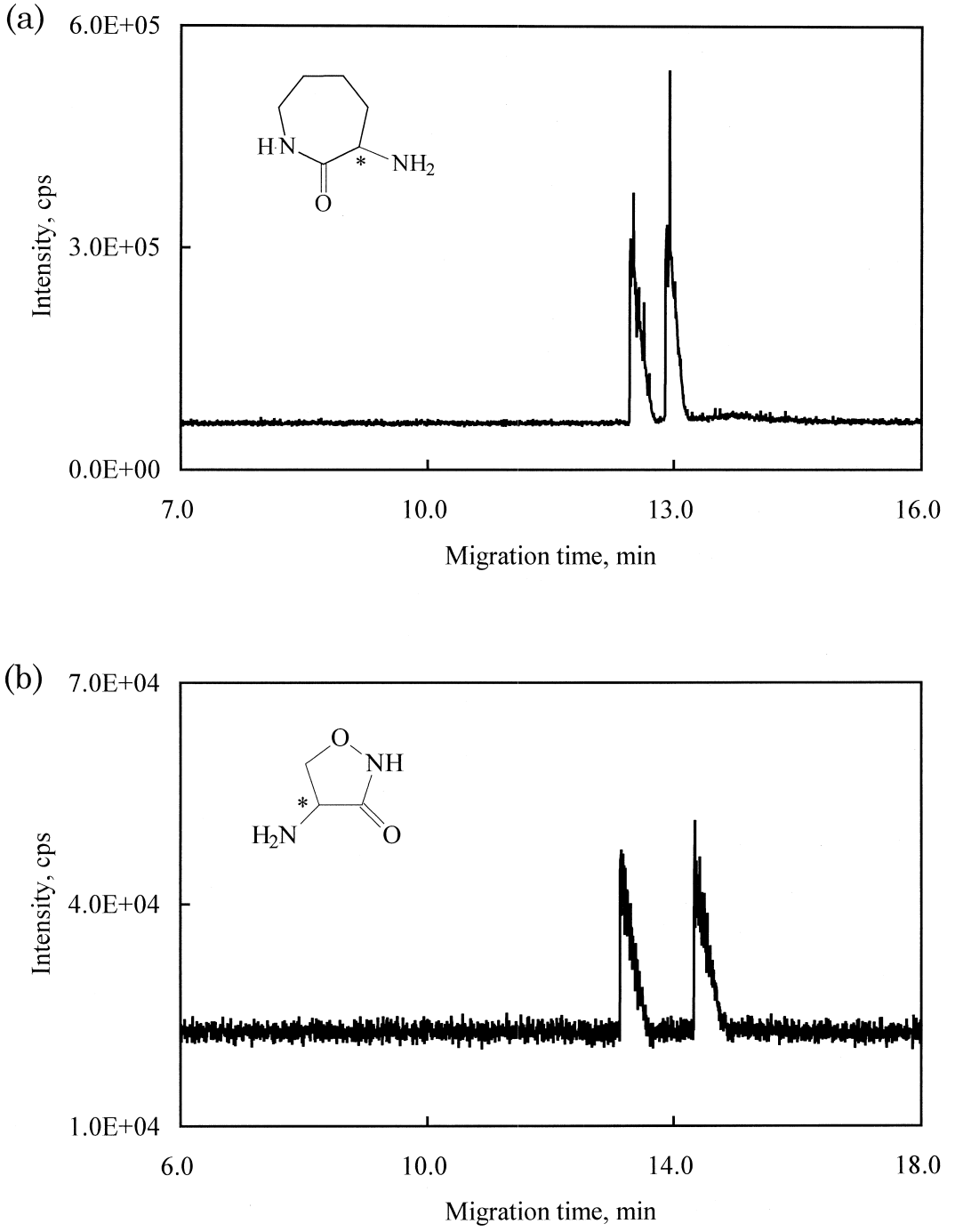

Fig. 5. Enantiomer separations of (a) racemic α-amino-ϵ-caprolactam and (b) racemic cycloserine. Conditions: sample concentration, 50 μg/ml; separation solution, 5 m*M* 18C6H_4 in the running buffer; MS detection, (a) *m*/*z* 129.1, (b) *m*/*z* 103.1 (positive ion mode). Other conditions are as in Fig. 4.

species to a volatile one, many enantiomers should be successfully separated by CE–MS.

Because the EOF is almost completely suppressed and $18C6H_4$ migrates towards the opposite direction of the MS detector, the contamination on the nozzle of the CE–MS interface and/or the orifice plate caused by the nonvolatile chiral selector is avoided even if the whole filling method is performed. However, the partial filling technique has an advantage that the contamination of $18C6H_4$ is also avoided during the washing of capillary with the separation solution prior to the run. Furthermore, uncharged chiral selectors can be also employed by this technique. Any types of chiral selectors can be used for the MS detection, unless they migrate in the same direction as the analytes. Thus CE–MS combined with the partial filling technique can be applied to enantiomer separations using a variety of chiral selectors.

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