ELSEVIER

Journal of Chromatography A, 802 (1998) 83–88

JOURNAL OF CHROMATOGRAPHY A

Separation of acidic enantiomers by capillary electrophoresis–mass spectrometry employing a partial filling technique

Yoshihide Tanaka[a,*], Yasuhiro Kishimoto[a], Shigeru Terabe[b]

[a]*Department of Analytical Chemistry, Nippon Boehringer Ingelheim Co. Ltd., 3-10-1, Yato, Kawanishi, Hyogo 666-01, Japan*
[b]*Faculty of Science, Himeji Institute of Technology, Kamigori, Hyogo 678-12, Japan*

Abstract

Enantiomer separations were performed by capillary electrophoresis–mass spectrometry (CE–MS). Because high concentrations of nonvolatile chiral selectors were not compatible for the CE–MS interface and/or the MS instrument, a partial filling technique was employed to avoid the problem. In this method, a separation solution containing a chiral selector was filled in only a part of the capillary. Since the electroosmotic flow (EOF) was almost completely suppressed in a polyacrylamide-coated capillary, neither the uncharged chiral selector nor the positively charged chiral selector migrated towards the MS detector under the negative polarity mode. Using either cyclodextrins (CDs) or avidin as a chiral selector, racemic mixtures of camphorsulfonic acid, tropic acid, arylpropionic acid and warfarin were successfully separated and detected by MS. This method was also applied to the optical purity test of commercial camphorsulfonic acid enantiomers.

Keywords: Enantiomer separation; Capillary electrophoresis–mass spectrometry; Partial filling capillary electrophoresis; Avidin; Camphorsulfonic acid

1. Introduction

Recently, capillary electrophoresis (CE) has become a powerful tool for the separation of enantiomers. A number of applications have been reported with various chiral selectors, such as cyclodextrins (CDs), proteins and macrocyclic antibiotics [1,2]. In particular, enantiomer separations are important in pharmaceutical science, and many papers have been published on the separation of drug enantiomers. CE instruments equipped with UV detectors are usually employed for the enantiomer separations because most drug enantiomers have strong UV absorption. In CE, since other detection methods are not popular, the separations of enantiomers having weak UV absorption have rarely been reported until now. Coupling of CE with mass spectrometry (MS) is a promising technique also for the detection of UV nonabsorbing enantiomers.

The development of CE–MS has been carried out by many groups since the first paper by Olivares and co-workers in 1987 [3]. Review articles containing more than 100 references have been published recently [4,5]. Several papers have reported on CE–MS of enantiomer separations [6–8]. A chiral selector must be added to a separation solution in order to obtain the enantiomer separation in CE, but most chiral selectors are not compatible with the CE–MS interface and/or with the MS instrument, due to their nonvolatility. Furthermore, high concentrations of the chiral selectors reduce the MS signal intensity of analyte ions. To solve these problems, Lamoree et al.

*Corresponding author

0021-9673/98/$19.00
PII S0021-9673(97)01073-X

[8] developed a heart-cut technique using the liquid junction method for the separation of ropivacaine racemate, where CD was removed before entering into the MS interface.

Some chiral selectors having strong UV absorption cause a deterioration in the detection of analytes by UV photometry, but the problem has been solved by using the partial filling technique [9–13] or the heart-cut technique [8,14]. In our previous study, we introduced the partial filling technique for enantiomer separations [10,11]. The partial filling technique was first developed by Valtcheva et al. in 1993 [9], and we modified the technique to run a commercial CE instrument automatically [10]. For CE–MS, this technique is also practically useful to avoid the introduction of the chiral selector to the MS instrument. In this paper, we describe separations of enantiomers, including weakly UV-absorbing ones, by on-line CE–MS employing the partial filling technique. This technique is also applied to the optical purity test of commercial camphorsulfonic acids.

2. Experimental

2.1. Apparatus

A Hewlett-Packard 3D CE instrument (Yokogawa Analytical Systems, Tokyo, Japan) was employed for the CE separation. The CE instrument control was performed with a Hewlett-Packard Vectra XM Series 3 (5/120) computer. A Perkin-Elmer Sciex API-300 triple quadrupole MS instrument (Perkin-Elmer Japan, Yokohama, Japan) was employed as the detector. The MS instrument control and data collections were performed with a Macintosh computer (model 8500/120). The pneumatically assisted electrospray (ion spray) interface supplied by Perkin-Elmer Sciex was employed for the coupling of CE and MS. A fused-silica capillary of 50 μm I.D.×150 μm O.D. (GL Science, Tokyo, Japan) was coated with linear polyacrylamide [15]. The capillary, of 80 cm in total length, was incorporated into a user-assembled capillary cartridge. For the delivery of a sheath liquid, a Harvard Apparatus syringe pump (Model 11, South Natick, MA, USA) was used.

2.2. Reagent

Racemic camphorsulfonic acid sodium salt, (*R*)-(−)-camphorsulfonic acid, (*S*)-(+)-camphorsulfonic acid, racemic tropic acid, racemic ibuprofen and racemic ketoprofen were purchased from Wako (Osaka, Japan). Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) was purchased from Nakalai Tesque (Kyoto, Japan), and quaternary ammonium β-cyclodextrin (QA-β-CD) was from Supelco (Bellefonte, PA, USA). Racemic warfarin was obtained from Tanabe Seiyaku (Osaka, Japan), and avidin was obtained from Eisai (Tokyo, Japan). All other reagents were of analytical or HPLC grade. Water was purified with a Milli-Q Labo system (Nihon Millipore, Yonezawa, Japan).

2.3. Procedure

For the optimization of MS conditions, a sample solution (ca. 50 μg/ml) was prepared in a mixture of 40 m*M* ammonium formate solution–methanol (1:1). The sample solution was infused into the ISP interface directly at 5 μl/min with a Harvard Apparatus syringe pump. The MS detection was performed in the negative ion mode. The ion spray voltage was maintained at −4.5 kV. MS conditions were optimized in order to produce the highest peak intensity of each quasi-molecular ion.

For the separation of camphorsulfonic acid enantiomers, 40 m*M* ammonium formate buffer (pH 4.0) was used as the running buffer. The separation solution was prepared by dissolving 50 m*M* DM-β-CD in the running buffer. For the separation of racemic tropic acid, 40 m*M* ammonium acetate buffer (pH 5.0) was used as the running buffer, and 5 m*M* QA-β-CD was dissolved in the running buffer to prepare the separation solution. For the separations of racemic ibuprofen and racemic ketoprofen, 40 m*M* ammonium acetate buffer (pH 6.0) containing 10% (v/v) 2-propanol was used as the running solution, and 100 μ*M* avidin was used as a chiral selector. As for the separation of warfarin racemate, 10% (v/v) ethanol was used as the organic modifier instead of 2-propanol mentioned above. All solutions were filtered through a 0.22-μm syringe-type membrane filter prior to use. A mixture of each running buffer–methanol (1:1) was used as a sheath liquid. The sheath liquid was delivered at 5 μl/min.

The principle of the partial filling technique was described in a previous paper [10]. Because the CE instrument and the total length of the capillary were different from that employed in the previous paper, the procedure is briefly described. At beginning and end of each day, the capillary was washed with the capillary wash solution (Bio-Rad, Cat. No. 148-5022) and water at 94 kPa (940 mbar) for more than 10 min each. The capillary was rinsed with the running buffer for 5 min at 94 kPa (940 mbar) prior to each run, and was filled with the separation solution at 5 kPa (50 mbar) 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 −30 kV was applied. The ion spray voltage was not applied during sample injection and for 1 min from each start of the run, and then −4.5 kV was applied at the other end of the capillary (the net voltage across the capillary was −25.5 kV). The MS detection was performed in the selected ion monitoring (SIM) mode for the negative quasi-molecular ion. After the capillary was rinsed with the following running buffer, the next analysis was performed continuously.

3. Results and discussion

3.1. Partial filling technique

The chiral selector may cause a decrease in ionization efficacy at the ion spray interface. Moreover, a high concentration of the chiral selector contaminates the nozzle of the interface and/or orifice plate. Therefore, we employed the partial filling technique in order to avoid the introduction of the chiral selector into the interface. First, we optimized the injection time or the length of separation solutions. The DM-β-CD solution was used as the separation solution in this experiment, and introduced continuously into the capillary at 5 kPa (50 mbar) after washing the capillary with the running buffer, and the chiral selector (DM-β-CD) eluting out from the end of the capillary was monitored by MS. The fragment peaks of DM-β-CD were detected at 21.8 min. Because the total length of the capillary was 80 cm, the length of the separation solution was calculated to be about 55 cm when it was injected for 15 min. When different separations or running solutions are employed, the length of the separation zone will be slightly different due to the difference in viscosity. However, the separations will not be affected significantly by the slight different in length of the separation zone. Therefore, the injection time of the separation solution was fixed at 15 min throughout this study. Because EOF in the coated capillary was almost completely suppressed, and the chiral selector, DM-β-CD, had no charge, the chiral selector hardly migrated during the analysis. The enantiomers, which migrated towards the MS detector, were separated when they passed through the separation solution. After passing through the separation solution, both enantiomers migrated at identical velocities in the running buffer. Accordingly, the chiral selector was not introduced into the interface. When the positively charged chiral selectors, such as cationic CDs and avidin, were used, the chiral selectors migrated towards the cathode or the injection end of the capillary, and were not introduced into the interface. This technique is applied to the separation using various chiral selectors, unless the chiral selectors migrate in the same direction as the analytes.

3.2. Enantiomer separation of racemic compounds

In CE–MS, a sheath liquid containing an organic solvent is commonly used to enhance the stability of the electrospray. When a mixture of each running solution–methanol (1:1) was used as the sheath liquid, the signal intensities of the analytes were sufficiently strong. Therefore, the composition of sheath liquid was not optimized any further in this study.

Fig. 1 shows the enantiomer separation of racemic camphorsulfonic acid. Because this compound has a weak UV absorption, detection by the UV detector is difficult. However, such analytes can be detected with high sensitivity by MS, thus CE–MS has an attractive feature for the detection of enantiomers having a weak UV absorption.

In CE, some charged chiral selectors, such as cationic CDs [16,17], proteins [18] and vancomycin [19], have been reported for the separation of acidic enantiomers. Oppositely charged chiral selectors were particularly effective for the charged enantiomer separations. The partial filling technique was

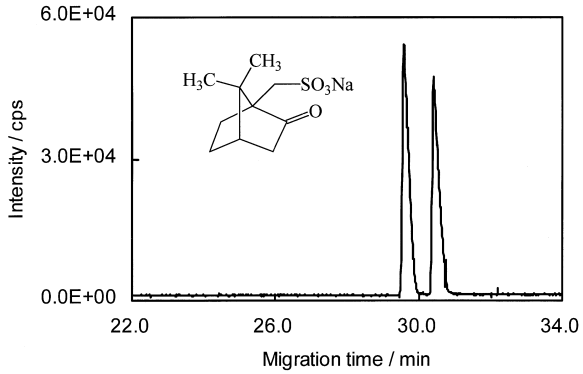

Fig. 1. Enantiomer separation of racemic camphorsulfonic acid by CE–MS. Conditions: sample solution, racemic camphorsulfonic acid sodium salt in water (50 μg/ml); sample injection, 50 mbar, 8 s; capillary, 80 cm×50 μm I.D. polyacrylamide-coated capillary; running buffer, 40 m*M* ammonium formate buffer (pH 4.0); separation solution, 50 m*M* DM-β-CD in the running buffer; sheath liquid, a mixture of the running buffer–methanol (1:1), 5 μl/min; voltage, −30−(−4.5) kV; detection, *m*/*z* 230.8 (negative ion mode); dwell time, 500 ms.

also applied to the separations of acidic enantiomers using QA-β-CD and avidin. Avidin is a basic egg-white protein having an isoelectric point (p*I*) value of ca. 10, and positively charged in a neutral buffer solution. The separation conditions previously reported [17,18] were employed for CE–MS by changing the buffer electrolytes from sodium phosphate to

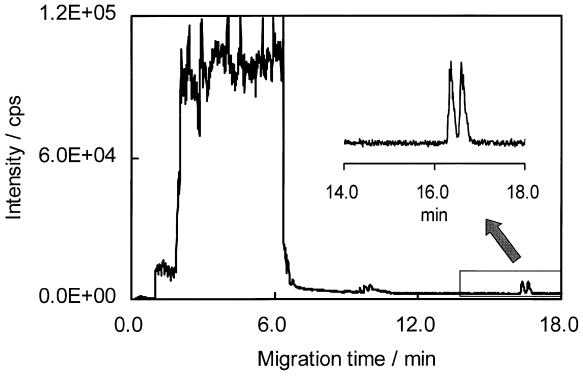

Fig. 2. Enantiomer separation of racemic tropic acid using QA-β-CD as a chiral selector. Conditions: sample solution, racemic tropic acid in a mixture of water–methanol (4:1), 50 μg/ml; running buffer, 40 m*M* ammonium acetate buffer (pH 5.0); separation solution, 5 m*M* QA-β-CD in the running buffer; detection, *m*/*z* 165.0 (negative ion mode); dwell time, 500 ms. Other conditions as in Fig. 1.

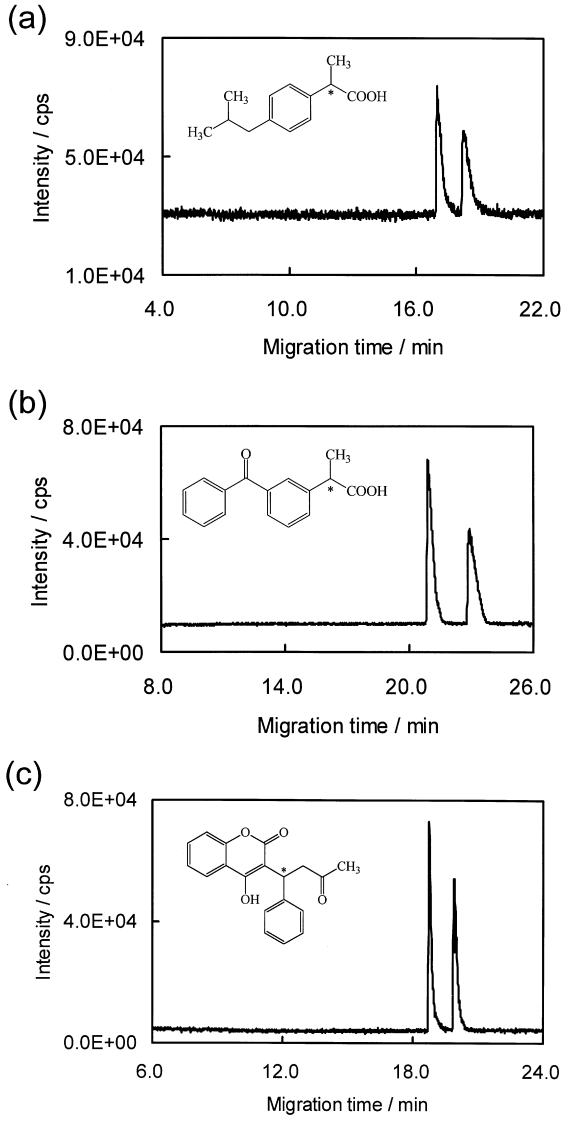

Fig. 3. Enantiomer separation of racemic arylpropionic acids and warfarin using avidin as a chiral selector. Conditions: sample solution, (a) racemic ibuprofen, (b) racemic ketoprofen and (c) racemic warfarin in a mixture of water–methanol (4:1), 50 μg/ml; running buffer, (a,b) 40 m*M* ammonium acetate buffer (pH 6.0) containing 10% (v/v) 2-propanol, (c) 40 m*M* ammonium acetate buffer (pH 6.0) containing 10% (v/v) ethanol; separation solution, 100 μ*M* avidin in the running buffer; detection, (a) *m*/*z* 205.0, (b) *m*/*z* 253.0 and (c) *m*/*z* 306.6 (negative ion mode); dwell time, 500 ms. Other conditions as in Fig. 1.

ammonium acetate, and enantiomer separations were successfully obtained as shown in Fig. 2 and Fig. 3. In Fig. 2, the baseline was disturbed until 7 min, probably due to the unstable electrospray caused by chloride ion. In fact, a large amount of chloride ion in QA-β-CD must have been introduced into the interface because the counter ion of QA-β-CD is chloride, and ca. 2% of sodium chloride was also included in QA-β-CD as an impurity [17]. When the analytes were detected, however, the baseline became stable. Fig. 3 shows that the partial filling technique is useful for the case of the protein chiral selector.

3.3. Optical purity test of camphorsulfonic acid enantiomer

In applications of enantiomer separations, the optical purity test is one of the important items. Therefore, we applied this method to the determination of optical purity of commercial camphorsulfonic acid enantiomers. The separation conditions were the same as those of the separation of camphorsulfonic acid racemate. As shown in Fig. 4, it was found that both enantiomers contained minor enantiomeric impurity. From the calculation of the ratio of the peak areas, the (*R*)-(−)-enantiomer included ca. 2.0% of the enantiomeric impurity. A signal-to-noise ratio of 14 was obtained for the enantiomeric impurity. For the commercial (*S*)-(+)-enantiomer, ca. 0.9% of impurity was detected, where the signal-to-noise ratio was 4.2. From these results, it was demonstrated that the detection sensitivity of CE–MS was sufficient to detect minor enantiomeric impurity.

4. Conclusion

Enantiomer separations were performed by CE–MS employing the partial filling technique. Several acidic enantiomers were successfully separated using DM-β-CD, QA-β-CD and avidin as chiral selectors. One of the merits of MS detection is that the analytes having weak UV absorption can be detected with high sensitivity. As for the optical purity test of camphorsulfonic acid enantiomer, from 1 to 2% of the enantiomeric impurity was easily detected. The detection sensitivity was practically enough to perform the optical purity test.

In CE, many applications of enantiomer separations have been reported using UV detection. By changing the buffer electrolyte from a nonvolatile species to a volatile one, these separation conditions will be transferred to this method. The type of chiral selectors does not restrict MS detection unless the chiral selectors migrate in the same direction as the analytes. Furthermore, separation solutions containing either ionic or nonionic surfactants are also employed in this method, as published elsewhere

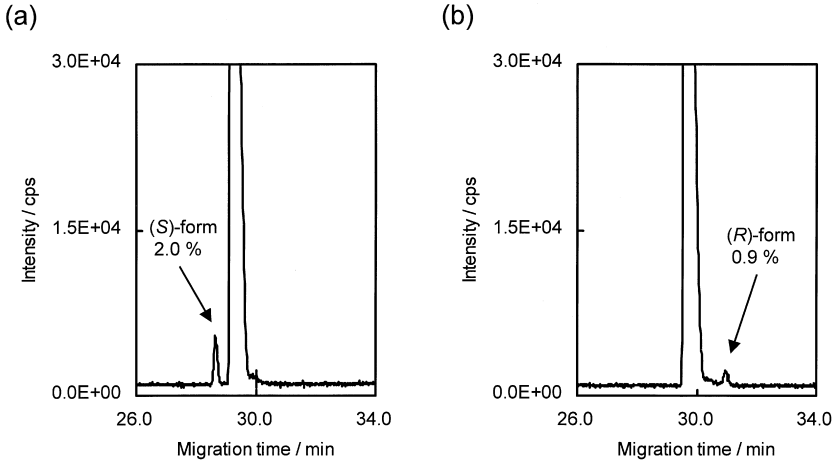

Fig. 4. Optical purity tests of camphorsulfonic acid enantiomers. Conditions: sample solution, (a) (*R*)-(−)-camphorsulfonic acid enantiomer and (b) (*S*)-(+)-camphorsulfonic acid enantiomer in water (100 μg/ml). Other conditions as in Fig. 1.

[20–22]. Thus, CE–MS combined with the partial filling technique can be applied to a wide range of the separations.

Acknowledgements

The authors gratefully acknowledge Dr. M. Miyagi (Takara Shuzo Co.) and Ms. K. Minoura (Yokogawa Analytical Systems) for their support on coupling the CE instrument to the API-300, and Dr. H. Nishi (Tanabe Seiyaku Co.) and Mr. Y. Ishihama (Eisai Co.) for the supply of the reagents.

References

[1] H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245.
[2] S. Fanali, J. Chromatogr. A 735 (1996) 77.
[3] J.A. Olivares, N.T. Nguyen, C.R. Yonker, R.D. Smith, Anal. Chem. 59 (1987) 1230.
[4] R.D. Smith, J.H. Wahl, D.R. Goodlett, S.A. Hofstadler, Anal. Chem. 65 (1993) 574A.
[5] J. Cai, J. Henion, J. Chromatogr. A 703 (1995) 667.
[6] R.L. Sheppard, X. Tong, J. Cai, J.D. Henion, Anal. Chem. 67 (1995) 2054.
[7] H.-J. Gaus, Z.Z. Gögüs, K. Schmeer, B. Behnke, K.-A. Kovar, E. Bayer, J. Chromatogr. A 735 (1996) 221.
[8] M.H. Lamoree, A.F.H. Sprang, U.R. Tjaden, J. van der Greef, J. Chromatogr. A 742 (1996) 235.
[9] L. Valtcheva, J. Mohammad, G. Pettersson, S. Hjertén, J. Chromatogr. 638 (1993) 263.
[10] Y. Tanaka, S. Terabe, J. Chromatogr. A 694 (1995) 277.
[11] Y. Tanaka, S. Terabe, Chromatographia 44 (1997) 119.
[12] F. Kilár, S. Fanali, Electrophoresis 16 (1995) 1510.
[13] T.J. Ward, C. Dann III, A.P. Brown, Chirality 8 (1996) 77.
[14] S. Krásenský, S. Fanali, L. Krivánková, P. Bocek, Electrophoresis 16 (1995) 968.
[15] M. Nakatani, A. Shibukawa, T. Nakagawa, Biol. Pharm. Bull. 16 (1993) 1185.
[16] A. Nardi, A. Eliseev, P. Bocek, S. Fanali, J. Chromatogr. 638 (1993) 247.
[17] Y. Tanaka, S. Terabe, J. Chromatogr. A 781 (1997) 151.
[18] Y. Tanaka, N. Matsubara, S. Terabe, Electrophoresis 15 (1994) 848.
[19] D.W. Armstrong, K.L. Rundlett, J.-R. Chen, Chirality 6 (1994) 496.
[20] W.M. Nelson, Q. Tang, A.K. Harrata, C.S. Lee, J. Chromatogr. A 749 (1996) 219.
[21] K. Koezuka, H. Ozaki, N. Matsubara, S. Terabe, J. Chromatogr. B 689 (1997) 3.
[22] P.G. Muijselaar, K. Otsuka, S. Terabe, J. Chromatogr. A, in press.