Journal of Chromatography A, 1218 (2011) 1775–1779

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A highly sensitive method for enantioseparation of fenoprofen and amino acid derivatives by capillary electrophoresis with on-line sample preconcentration

Zhaoyan Wang [a,b], Chao Liu [a], Jingwu Kang [a,*]

[a] Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Lingling Road 345, Shanghai 200032, China
[b] College of Pharmaceutical sciences, Lanzhou University, Lanzhou 730000, China

ARTICLE INFO

Article history:
Received 25 August 2010
Received in revised form 26 January 2011
Accepted 27 January 2011
Available online 4 February 2011

Keywords:
Enantioseparation
Capillary electrophoresis
Large-volume sample stacking

ABSTRACT

A highly sensitive method for enantioseparation of trace fenoprofen and amino acid derivatives by capillary electrophoresis (CE) with vancomycin as the chiral selector was developed. Several CE techniques, such as the partial filling, large-volume sample stacking with EOF as pump plus anion-selective exhaustive injection (LVSEP–ASEI) were involved in the present method to improve the detection sensitivity. With on-column concentration, enantioseparation of racemic fenoprofen and six 9-fluorenylmethyl chloroformate (FMOC)-amino acid derivatives (at the concentration level of ng/mL) with the background electrolyte composed of 100 mmol/L Tris–H_3PO_4 (pH 6.0) and 2 mmol/L vancomycin was detected readily with the UV detection at 214 nm. Successfully performing LVSEP–ASEI needs a very low EOF that could be depressed by coating the capillary with poly(dimethylacrylamide) solution. The coating also played a role to minimize the adsorption of vancomycin onto the capillary wall. Effect of the injected sample volume and the electrokinetic injection time on the peak area of the enantiomers and their resolution factor were investigated and optimized. Under the optimized conditions, more than 1000-fold enhancement in detection sensitivity compared with the normal injection was achieved.

1. Introduction

Capillary electrophoresis (CE) has become one of the most powerful tools for enantioseparation due to its advantages such as high separation efficiency, low operational cost, simplicity and versatility [1–5]. However, poor concentration sensitivity due to the small injection volume and short optical path length is considered to be a major limitation. To overcome this shortcoming several kinds of on-column sample concentration techniques, such as large volume sample stacking (LVSS) or field-amplified sample injection [6,7], sweeping technique [8,9], anion or cation-selective exhaustive injection [10,11], and pH-mediated field-amplified sample stacking [12] have been developed to enhance the detection sensitivity of CE [13–16]. The detection sensitivity of CE can be further improved by combining two sample concentration techniques, for example, sweeping plus large volume sample stacking, large-volume sample stacking using EOF pump with anion-selective exhaustive injection (LVSEP–ASEI), etc. [11,17,18].

According to a recent statistics by using SciFinder, more than 2600 papers concerning enantioseparation by CE have been published in the international journals since the first report of enantioseparation by CE published in 1985 [19]. Among these papers, a few addressed the effort to improve the detection sensitivity when enantioseparation with CE was studied [20–23]. On-column concentration techniques, such as sweeping and stacking were applied for enantioseparation of triadimenol by Otsuka et al. [20]. About 10-fold enhancement in the detection sensitivity was achieved. A CE method with laser-induced fluorescence detection for the enantioseparation of amino acids derivatives of cyanobenzisoindole (CBI) was developed with sulfated β-CD (S-β-CD) as the chiral selector at low pH. About 100-fold improvement in signal-to-noise ratio was obtained. The authors proposed that the on-column concentration process consists of field-amplified migration, pH-mediated stacking, and sweeping by S-β-CD. The obtained limits of detection for CBI-DL-serine and CBI-DL-glutamate were 0.20 and 0.30 nmol/L, respectively [21]. Quirino and Terabe demonstrated that the separation of low concentration racemic herbicide fenoprop, which was spiked in the lake water, could be achieved by MEKC sweeping and γ-CD as the chiral selector. The detection sensitivities as low as 9 ppb (first eluted peak) and 18 ppb (second peak) were obtained [22]. A CE method for enantioseparation and detection of six beta-blocker enantiomers using carboxymethyl-β-CD as the chiral selector was developed by Huang et al. [23]. With the aid of the field-amplified sample injec-

Abbreviations: LVSEP, large-volume sample stacking with EOF as pump; ASEI, anion-selective exhaustive injection; PDMA, poly(N,N-dimethylacrylamide); APS, ammonium persulfate; TEMED, N,N,N′,N′-tetramethylethyl-enediamine.

* Corresponding author. Tel.: +86 21 54925385; fax: +86 21 54925385.
E-mail address: jingwu.kang@sioc.ac.cn (J. Kang).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.01.081

tion, enantiomers as low as 0.01 mg/mL were able to be separated and detected.

Vancomycin has been considered as a highly selective chiral selector in CE [24]. In our previous work, its separation performance was dramatically improved using the coated capillary with poly(dimethylacrylamide) (PDMA) [25]. In the present work, we try to improve the detection sensitivity of this highly selective method so that it can be used to separate enantiomers at very low concentration (ng/mL). A potential application of this highly sensitive enantioseparation method may be in the field of clinical analysis of chiral drug or in the field of environment analysis. It has been reported that the ratio of enantiomers of a racemic chiral drug like ibuprofen in sewage changed remarkably after treatment in the wastewater treatment plant [26,27]. Therefore, the chiral drugs in sewage can be used as a marker for judgment of the inefficient wastewater treatment and direct discharges of untreated wastewater. It has been reported in the literature that the concentration of chiral drugs in river or wastewater was around ng/mL level [26]. GC–MS instrument and the time-consuming sample derivative were needed, so as to detect such a low concentration of enantiomers [26].

The aim of the present paper is to try to develop a sensitive method for detection of enantioseparation of environmental sample by CE. Acidic enantiomers including fenoprofen and six FMOC-amino acid derivatives were employed as the model compounds for experimental design. By using LVSEP–ASEI, the enantioseparation of fenoprofen as low as 0.38 ng/mL for each enantiomer in pure water could be detected. There is almost 1000-fold enhancement in detection sensitivity compared with the normal injection. Racemic fenoprofen spiked in river water sample at the concentration of 125 ng/mL (62.5 ng/mL for each enantiomers) could be detected with our method.

2. Experimental

2.1. Instrumentation

All separations were performed on a Beckman P/ACE MDQ CE system (Fullerton, CA, USA) equipped with a UV detector. Data collection, processing, and analysis were performed using system 32-Karat software (Beckman). Fused silica capillary with a dimension of 50 μm i.d. and 370 μm o.d. was purchased from Yongnian Optical Fiber Inc. (Hebei, China). The total length of the capillary was 39 cm and the effective length was 29 cm. The capillary was thermostated at 20 °C. Samples were detected with UV 214 or 254 nm. Voltages of −5 kV and −15 kV were applied for electrokinetic injection and separation, respectively.

2.2. Reagents

Amino acids, fenoprofen, and 9-fluorenylmethyl chloroformate (FMOC) were purchased from Sigma (St. Louis, MO, USA). N,N-dimethylacrylamide (DMA) was obtained from Aldrich (Steinheim, Germany). N,N,N′,N′-tetramethylethyl-enediamine (TEMED) was obtained from Fluka (Buchs, Switzerland). Tris(hydroxymethyl) aminomethane (Tris) and ammonium persulfate (APS) were from Shanghai Shisheng Biotechnological Inc. (Shanghai, China). Vancomycin hydrochloride was obtained from North China Pharmaceutical Co. All reagents were of analytical grade. Stock solutions of Tris (500 mmol/L) and phosphoric acid (1 M) were prepared. Background electrolyte composed of 100 mmol/L Tris (pH 6.0) in the presence or absence of 2 mmol/L vancomycin was prepared from the stock solutions. The pH of the background electrolyte was adjusted with the solution of phosphoric acid to a desired value. All solutions were prepared with the deionized water prepared by ultrapure water system (Millipore, MA, USA) and filtered through a 0.45-μm nylon filter prior to use.

2.3. Procedure

Derivatization of amino acids with FMOC was performed according to the protocol described in the literature [28]. Briefly, 200 μL of 10 mmol/L FMOC solution in acetonitrile was added to 200 μL of 3 mmol/L amino acid in 100 mmol/L borate buffer (pH 9.3). After reaction for 2 min, the mixture was extracted with 0.5 mL pentane twice to remove the excess of the derivative reagent. After dilution to a desired concentration with water, the sample was ready for analysis.

PDMA was synthesized in the laboratory according to the literatures [22–24]. Briefly, the reaction solution comprising 1.4 mol/L DMA in water was degassed ultrasonically for 15 min, and polymerization was initiated by adding 10% (m/v) APS solution to a final concentration of 4 mg/mL and TEMED solution to a final concentration of 4 mg/mL. Polymerization was allowed to continue for 24 h at room temperature. The un-reacted monomer was removed by dialyzed against water using a 12-kDa cut-off dialysis membrane. The high relative molecular mass polymer was precipitated by addition of acetone to the polymer solution and dried under vacuum. To prepare the coating solution, 0.2 g dry polymer was dissolved in 10 mL water and stirring for 24 h until a homogenous solution was obtained.

A new capillary was pretreated with 1 M NaOH for 30 min, followed by flushing the column with water and 0.5 M HCl for 5 min, respectively. Afterwards, the polymer solution was loaded into the capillary by pressure at 1.38 bar for 5 min. After keeping the polymer solution in the capillary for another 5 min, the excess gel solution was removed by flushing the capillary with the background electrolyte for 15 min by pressure of 1.38 bar. The resulting coating was proved to be stable enough for one hundred runs without significant change on the EOF. The coating can be easily removed by flashing the capillary with 1 M NaOH solution for 15 min. Then the capillary can be re-coated with the polymer solution.

2.4. LVSEP–ASEI

First of all, the capillary was fully filled with the background electrolyte containing 2 mmol/L vancomycin followed by injection of the sample by the pressure of 34.45 mbar for 8 min to give a 32 cm long sample plug in the capillary. More analyte was further injected with the anion-selective exhaustive injection (ASEI) by applying voltage of −5 kV for 12.5 min during the process of LVSEP. Subsequently, the inlet end of the capillary was switched to the vials containing 100 mmol/L Tris–phosphate buffer (pH 6.0) in the absence of vancomycin, and voltage of −15 kV was thereafter applied for the separation.

3. Results and discussion

In our previous work, high performance enantioseparation by CE with vancomycin as the chiral selector was achieved by dynamically coating the capillary with poly(dimethylacrylamide) (PDMA) combined with the partial filling technique [25]. These conditions were directly adopted in the present work except the concentration of the background electrolyte. In the present experiments, it was found that the stacking efficiency increased with increase of concentration of background electrolyte until 100 mmol/L. Therefore, 100 mmol/L Tris was fixed for the following experiments.

The experimental schedule for the large volume sample stacking and subsequent enantioseparation is shown in Fig. 1. Before

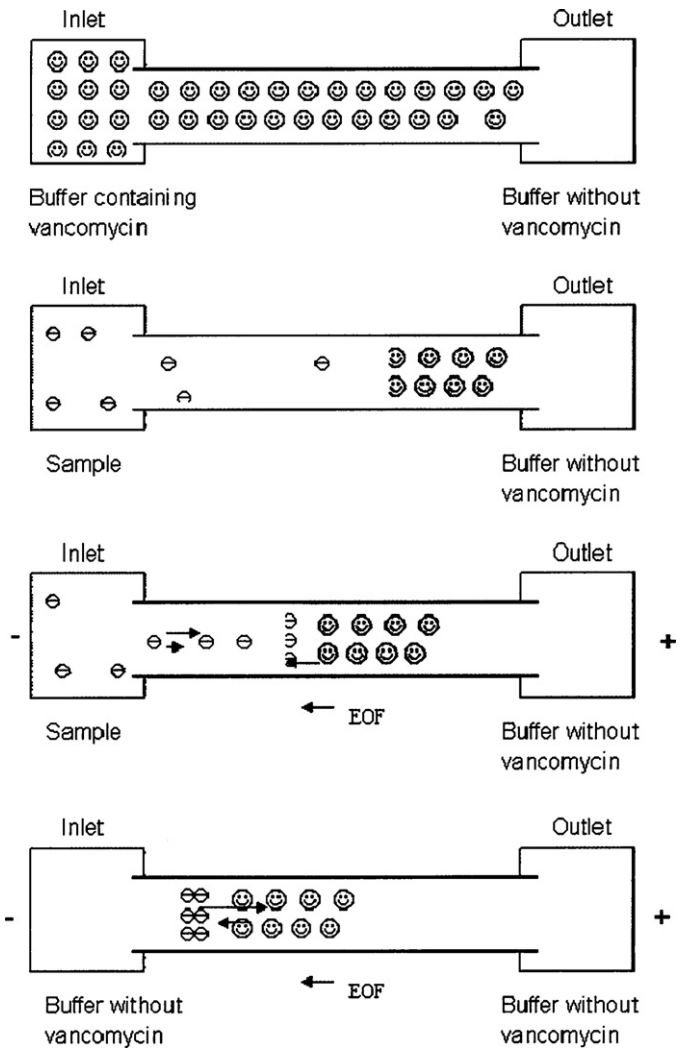

Fig. 1. Schematic representation for LVSEP–ASEI and enantioseparation. (1) Filling the capillary with the buffer containing 2 mmol/L vancomycin; (2) hydrodynamic injection of the sample by pressure; (3) stacking the sample and removing the water plug by EOF, and injection of more sample by ASEI; (4) enantioseparation.

injection, the capillary was fully filled with the buffer containing 2 mmol/L vancomycin. Large volume of sample was thereafter injected to leave a short plug of the background electrolyte in the outlet end of the capillary. The injected sample plug length can be precisely controlled by the injection time under a certain injection pressure. The linear velocity of the sample solution passing through the capillary was measured by recording the amount of time spent for appearance of a baseline drop due to the replacement of the vancomycin containing buffer (high UV absorbance) by the sample solution (UV transparency). In the present experiment, the linear velocity of the sample solution passing through the column was determined as 4.0 cm/min under the injection pressure of 34.45 mbar.

In the process of large volume sample stacking, the local EOF velocity in the sample plug will be greater than that in the background electrolyte plug. This is because almost all the applied voltage is distributed over the sample zone due to its much higher resistivity [9]. This will lead to a mismatch in the EOF velocities and result a laminar flow along the column, which will broaden the sharp sample zone already formed by sample stacking and destroy the resolution [29]. Such a problem can be reduced by pumping the water plug out of the capillary in the process of sample stacking by the counter-current EOF [30,31]. It should be noted that, the

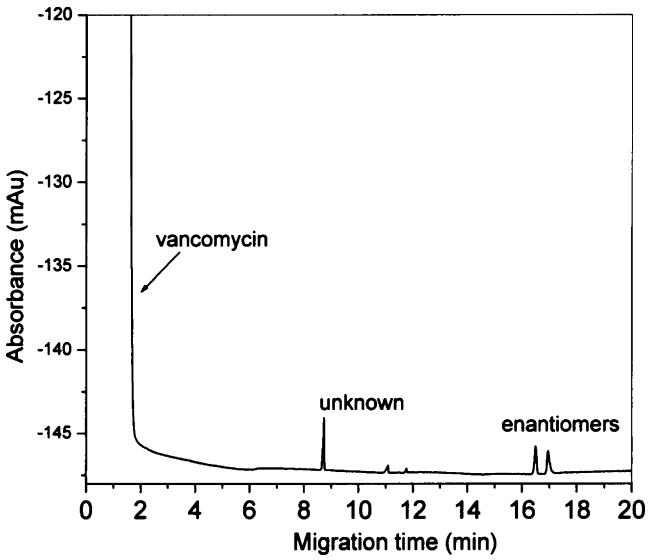

Fig. 2. Electropherogram for enantioseparation of FMOC-Ala after sample concentration with LVSEP–ASEI. Conditions: fused silica capillary, 50 μm i.d. (370 μm o.d.) × 39 cm (29.0 cm to detection window); background electrolyte for electrokinetic injection and separation, 100 mmol/L Tris–H_3PO_4 (pH 6.0) containing 2 mmol/L vancomycin was used for filling the capillary before injection; solution of enantiomers of FMOC-Ala (13.5 ng/mL) was injected by pressure at 34.47 mbar for 8 min, followed by electrokinetic injection with −5 kV voltage for 12.5 min detection wavelength, 214 nm; column temperature, 20 °C.

velocity of the counter-current EOF must be lower than the velocity of the analytes to avoid the analytes being pumped out of the capillary. Therefore, properly controlling the velocity of the EOF is very important for sample pre-concentration with LVSS technique. Addition of the organic modifier in the background electrolyte or use of acidic buffer was proved to be the effective way to suppress the EOF for such a purpose [30,31]. However, in our case, both methods were not compatible with the enantioseparation conditions. Therefore, dynamically coating the capillary with PDMA will be the best choice, because the coating not only suppress the EOF, but also diminish the adsorption of vancomycin onto the capillary wall. After coating the capillary with PDMA solution, the EOF measured with the method suggested by Williams and Vigh [32] was in the range from 1×10^{-9} to 9×10^{-9} $m^2/V\,s$ when buffer pH changed

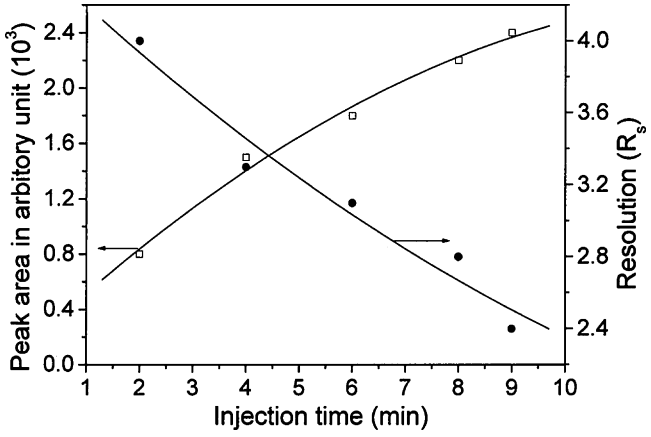

Fig. 3. Effect of the sample injected time on peak area and separation resolution. Conditions: background electrolyte 100 mmol/L Tris–H_3PO_4 (pH 6.0), the same buffer in the presence of 2 mmol/L vancomycin was used for filling the capillary before sample injection; solution of fenoprofen enantiomers (0.25 μg/mL for each enantiomer) were injected by the pressure of 34.47 mbar for various periods of time; applied voltage for separation, −15 kV.

from 4 to 8. It was reduced by 10-fold compared with the bare silica column.

Fig. 2 shows the electropherogram for enantioseparation of FMOC-Ala (13.5 ng/mL for each enantiomers) obtained after on-column pre-concentration by LVSEP–ASEI. In this electropherogram, the very large peak is due to the zone of vancomycin which moved towards the injection end of the capillary in the process of LVSEP [33] and the separation, while the analytes migrate oppositely to the anode (detection end). This makes possible to avoid the interference of the detection of vancomycin due to its strong UV absorbance. The unknown peaks in the electropherogram could come from FMOC.

In this experiment, the peak area of the first eluted peak of fenoprofen enantiomers was used as a criterion for optimizing the conditions of LVSEP–ASEI. Fig. 3 shows the effect of the injection time (corresponding to the injected sample volume) on the peak area of the diluted solution of fenoprofen (5 ng/mL for each enantiomer). It can be seen that the peak area increased, while the resolution factor decreased with increase of the injection time. This is because the longer the injection time the shorter the plug length of the background electrolyte is, while the resolution factor is proportional to the plug length of the vancomycin solution [26]. Therefore, a balance must be made between the sample enrichment and the resolution of enantioseparation. The minimum plug length of the background electrolyte for baseline separation of all tested enantiomers was determined as 7.0 cm. Therefore, the maximum injection length of diluted samples of enantiomers is about 32 cm (total length of the capillary is 39 cm) corresponding to the injection time of 8 min.

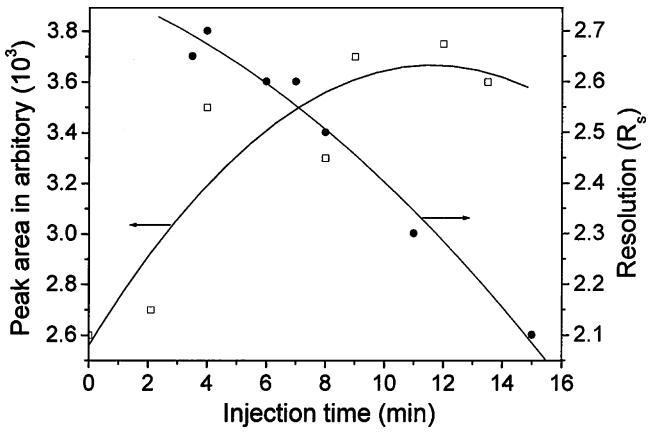

Fig. 4. Effects of the electrokinetic injection time on peak area and separation resolution. Conditions: background electrolyte for electrokinetic injection and separation, 100 mmol/L Tris–H_3PO_4 (pH 6.0), the same buffer in the presence of 2 mmol/L vancomycin was used for filling the capillary before sample injection; solution of fenoprofen enantiomers (0.25 μg/mL for each enantiomer) was injected by pressure at 34.47 mbar for 8 min, followed by electrokinetic injection with −5 kV voltage for various periods of time; other conditions as in Fig. 2.

Additional analyte can be selectively injected with electrokinetical injection by applying voltage of −5 kV in the process of LVSEP. As shown in Fig. 4, the peak area of the first eluted peak of enantiomers of fenoprofen increased with increase of the electrokinetic injection time until the appearance of a maximum value at 12.5 min, thereafter the peak area did not increase with increase of the injection time, probably because the analyte in sample solution was almost

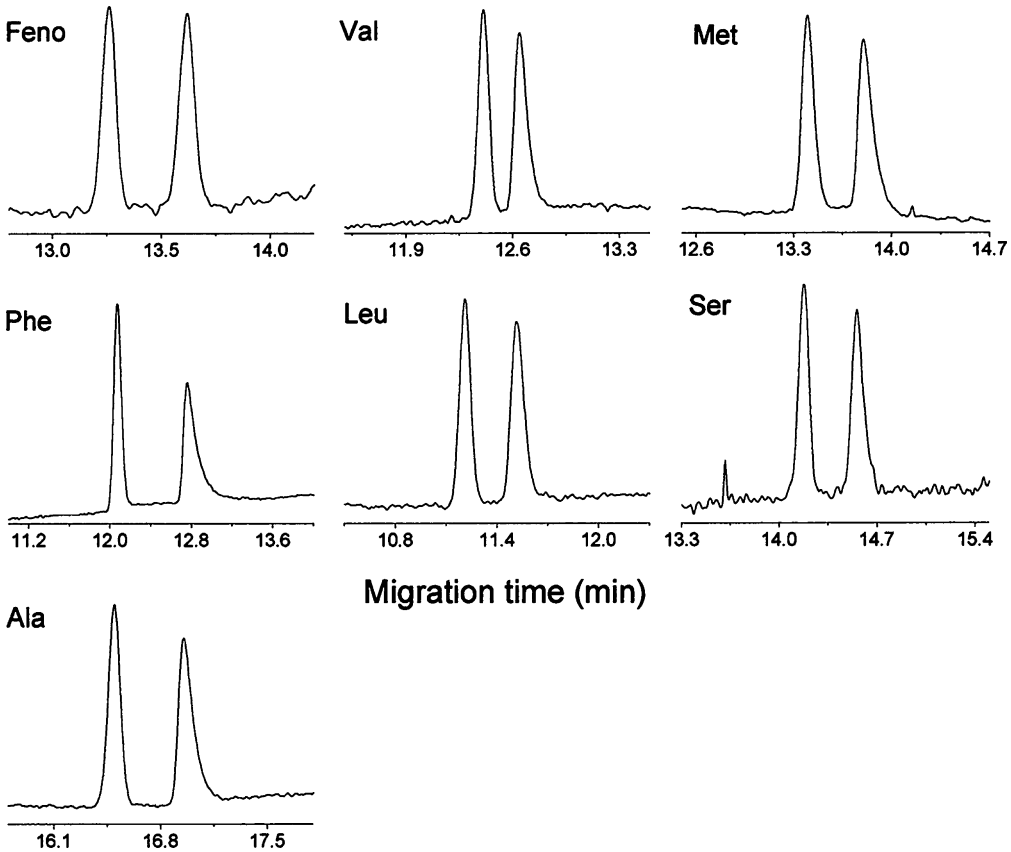

Fig. 5. Electropherograms of enantioseparation obtained after LVSEP–ASEI combined with the part filling technique. Conditions: background electrolyte for electrokinetic injection and separation, 100 mmol/L Tris–H_3PO_4 (pH 6.0), the same buffer in the presence of 2 mmol/L vancomycin was used for filling the capillary before injection; samples was injected by pressure at 34.47 mbar for 8 min, followed by electrokinetic injection by applying voltage of −5 kV for 12.5 min; concentrations for each enantiomer: fenoprofen, 2.5 ng/mL, FMOC-amino acids, 13.5 ng/mL; other conditions as in Fig. 2.

Table 1
Data for quantitative analysis and repeatability of the method.

Compounds	LOD (ng/mL)	LOQ (ng/mL)	Repeatability of migration time and peak height RSD (%) ($n = 6$)				
			t_1	t_2	H_1	H_2	R_s
Fenoprofen	0.38	1.25	0.88	0.83	2.79	2.99	3.90
Val	1.10	3.70	5.10	5.04	3.86	2.24	7.80
Ala	1.90	5.70	6.45	6.24	3.94	6.08	3.11
Phe	1.57	5.24	4.22	4.01	2.53	6.40	4.31
Ser	2.10	6.30	2.02	2.00	2.75	4.85	3.44
Met	1.07	3.58	3.27	3.26	3.93	4.37	3.01
Leu	1.48	4.91	2.48	2.46	4.85	5.00	2.07

Notes: t_1, t_2: migration times of the first and second eluted peaks of the enantiomers; H_1 and H_2: the peak heights of the first and second eluted peaks of the enantiomers; R_s: resolution factor. Conditions as in Fig. 5.

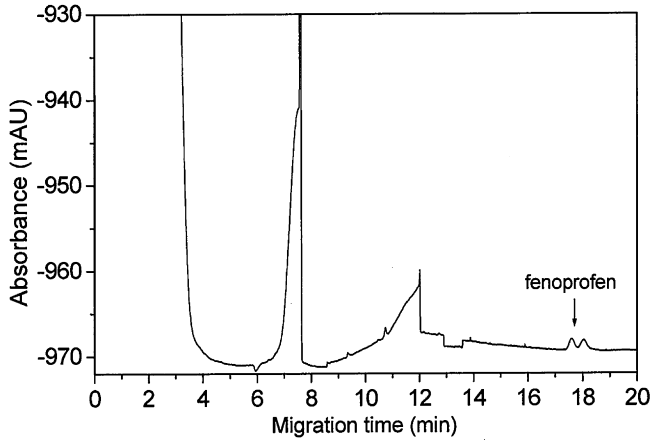

Fig. 6. Electropherogram for enantioseparation of 125 ng/mL fenoprofen in a river water sample obtained after LVSEP–ASEI combined with the part filling technique. Conditions are the same as in Fig. 5.

exhausted. On the other hand, the separation resolution decreased with increase of the electrokinetic injection time. This could be due to the fact that the prolonged sample band will decrease the resolution factor. Finally, the optimal electrokinetic injection time was determined as 12.5 min.

Electropherograms for enantioseparation obtained after on-column sample concentration by LVSEP–ASEI are shown in Fig. 5. Both high detection sensitivity and high resolution were obtained under the optimized conditions. The data for quantitative analysis and the repeatability in terms of the migration times and the peak heights are summarized in Table 1. For all tested enantiomers, the limit of detection in the range from 0.38 to 2.10 ng/mL was achieved.

A river water sample in which the enantiomers of fenoprofen were spiked was used for evaluating the practice application of the present method. The river water sample was filtered with 0.45-μm nylon filter, then spiked with racemic fenoprofen to a final concentration of 62.5 ng/mL for each enantiomers. As shown in Fig. 6, the baseline in the electropherogram was poor due to the complex matrix of the river water. Moreover, compared with the sample prepared with pure water, the enrichment efficiency was reduced by 10-fold. This could be due to the fact that relative high salt concentration in the river water.

4. Conclusions

A highly sensitive CE method for enantioseparation with CE has been developed. CE techniques, including LVSEP–ASEI and the partial filling technique were employed for sample pre-concentration and enantioseparation. It was proved that dynamically coating the capillary with polymer PDMA played a key role to suppress the EOF and simultaneously to diminish the adsorption of vancomycin onto the capillary wall. Under the optimized conditions, almost 1000-fold enhancement in detection sensitivity compared with the normal injection was achieved. The method could be potentially used for the analysis of the chiral drugs in environment sample and clinical samples.

Acknowledgement

This work was financially supported by the National Natural Science Foundations of China (90713021, 20975109 and 20675086).

References

[1] S. Fanali, Electrophoresis 30 (2009) S203.
[2] B. Chankvetadze, J. Chromatogr. A 1168 (2007) 45.
[3] B. Chankvetadze, Electrophoresis 30 (2009) S211.
[4] B. Preinerstorfer, M. Lammerhofer, W. Lindner, Electrophoresis 30 (2009) 100.
[5] P. Mikus, K. Marakova, Electrophoresis 30 (2009) 2773.
[6] D.S. Burgi, Anal. Chem. 65 (1993) 3726.
[7] R.L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 489A.
[8] J.P. Quirino, Y. Iwai, K. Otsuka, S. Terabe, Electrophoresis 21 (2000) 2899.
[9] J.P. Quirino, S. Terabe, Anal. Chem. 71 (1999) 1638.
[10] J.P. Quirino, S. Terabe, Anal. Chem. 72 (2000) 1023.
[11] C.D.S. Kim Bojeong, Electrophoresis 23 (2002) 49.
[12] D.J. Weiss, K. Saunders, C.E. Lunte, Electrophoresis 22 (2001) 59.
[13] S.L. Simpson Jr., J.P. Quirino, S. Terabe, J. Chromatogr. A 1184 (2008) 504.
[14] Z. Mala, A. Slampova, P. Gebauer, P. Bocek, Electrophoresis 30 (2009) 215.
[15] L.A. Kartsova, E.A. Bessonova, J. Anal. Chem. 64 (2009) 326.
[16] L. Sanchez-Hernandez, C. Garcia-Ruiz, M.L. Marina, A.L. Crego, Electrophoresis 29 (2008) 237.
[17] L. Zhu, H.K. Lee, Anal. Chem. 73 (2001) 3065.
[18] X.H. Zang, C.R. Li, Q.H. Wu, C. Wang, D.D. Han, Z. Wang, Chin. Chem. Lett. 18 (2007) 316.
[19] E. Gassmann, J.E. Kuo, R.N. Zare, Science 230 (1985) 813.
[20] K. Otsuka, M. Matsumura, J.-B. Kim, S. Terabe, J. Pharm. Biomed. Anal. 30 (2003) 1861.
[21] D.L. Kirschner, M. Jaramillo, T.K. Green, Anal. Chem. 79 (2007) 736.
[22] J.P. Quirino, S. Terabe, Science 282 (1998) 465.
[23] L. Huang, J.-M. Lin, L. Yu, L. Xu, G. Chen, Electrophoresis 29 (2008) 3588.
[24] W. Armstrong Daniel, L. Rundlett Kimber, J.-R. Chen, Chirality 6 (1994) 496.
[25] Z. Wang, J. Wang, Z. Hu, J. Kang, Electrophoresis 28 (2007) 938.
[26] H.-R. Buser, T. Poiger, M.D. Muller, Environ. Sci. Technol. 33 (1999) 2529.
[27] L.J. Fono, D.L. Sedlak, Environ. Sci. Technol. 39 (2005) 9244.
[28] H. Wan, P.E. Andersson, A. Engstroem, L.G. Blomberg, J. Chromatogr. A 704 (1995) 179.
[29] R.L. Chien, D.S. Burgi, J. Chromatogr. 559 (1991) 153.
[30] D.S. Burgi, R.L. Chien, Anal. Chem. 63 (1991) 2042.
[31] Y. He, H.K. Lee, Anal. Chem. 71 (1999) 995.
[32] B.A. Williams, G. Vigh, Anal. Chem. 68 (1996) 1174.
[33] R.L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 1046.