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Capillary electrochromatographic enantioseparations using a packed capillary with a 3 μm OD-type chiral packing

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Abstract

Enantiomer separations by capillary electrochromatography (CEC) using a packed capillary were investigated. As a chiral stationary phase, an OD-type packing material of 3 μm particle diameter, which is a silica-gel based material coated with cellulose tris(3,5-dimethylphenylcarbamate), was employed. The chiral packing was packed into a 100 μm I.D. fused-silica capillary by a pressurized method. Several racemic enantiomers were successfully resolved with acidic or basic buffers containing acetonitrile as mobile phases. The separation efficiencies obtained in this chiral CEC system were superior to those in the previously reported chiral CEC system using 5 μm particles. The typical plate heights obtained for several enantiomers were 4.6–6.6 μm or reduced plate heights of 1.5–2.2. Good repeatabilities in the migration time, peak height, and corrected peak area were observed as well as for the plate number. As an application study of this CEC system, an optical purity test was carried out by using benzoin enantiomers. A 1% content of one enantiomer in the other enantiomer was successfully separated and detected.

Keywords: Electrochromatography; Enantiomer separation; Capillary columns; Chiral stationary phases, electrochromatography; Benzoin

1. Introduction

Capillary electrochromatography (CEC) has become popular in recent years as a highly efficient separation technique utilizing an electromigration as well as capillary electrophoresis (CE) [1]. The application area of these techniques has gradually been increased owing mainly to the high efficiency, small quantity of samples, and short analysis time. Among the applications, enantiomer separation is one of important objectives of CEC and an increasing numbers of reports on chiral separations by CEC have appeared. In chiral separations by CEC, packed capillaries [2–10] with several chiral stationary phases (CSPs) and open-tubular capillaries [11–18] are normally used. In the former, the CSPs include the immobilized chiral moieties on silica supports and molecularly imprinted polymers, whereas in the latter the inside wall of the capillary is modified with appropriate agents to have an enantioselectivity, e.g., by dynamically coating with proteins.

Various types of CSPs are available for high-performance liquid chromatography (HPLC), whereas only a few CSPs have appeared for the use in CEC. We have reported the use of an OD-type chiral packing of 5 μm particle size, which has been originally prepared for the use in HPLC, in CEC and some enantiomers were optically separated with higher efficiency than that obtained in conventional HPLC [6]. In this paper, the use of a 3 μm particle

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OD-type packing as a CSP for enantiomer separation by CEC is investigated to achieve higher efficiency in comparison with the previous results using 5 μm particles. Some parameters affecting column performance, such as the velocity of the electroosmotic flow (EOF), the content of acetonitrile in a mobile phase, and column temperature, are briefly examined. Repeatabilities of the plate number, migration time, peak height, and corrected peak area in this system are also investigated. An application of this system to the optical purity test is also attempted by using benzoin enantiomers as test solutes.

2. Experimental

2.1. Materials and apparatus

The chiral packing used was an OD-type material (Daicel, Tsukuba, Ibaraki, Japan), which is a silica gel based 3 μm particle coated with cellulose tris(3,5-dimethylphenylcarbamate). A fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 24 cm effective length×100 μm I.D.×375 μm O.D. packed with the OD-type material was used as a separation capillary. Several racemic enantiomers, the names and chemical structures given in Fig. 1, were used as test solutes. Sample solutions were prepared by dissolving each enantiomer in a mobile phase at 0.1 mg/ml, except for the use in the optical purity test. Mobile phases were prepared by mixing a buffer solution with an appropriate amount of acetonitrile. Thiourea was used as a marker of a non-retained solute or the EOF, that is, the retention time of thiourea is considered as t_0. All chemicals were the highest grade available and used without further purification.

An Agilent Technologies CE system (Waldbronn, Germany) equipped with a UV detector controlled by a ChemStation software on Windows NT was used as a CEC instrument. Both inlet and outlet vials were pressurized at 10 bar with nitrogen to suppress the bubble formation inside the capillary during a CEC separation. As a syringe pump, a Harvard Apparatus Model 11 (South Natick, MA, USA) was used.

2.2. Procedure for packing

The procedure of the pressurized packing was similar to that reported previously [6] with a slight modification: (1) a *temporary frit* was made at an appropriate position, e.g., a few cm, from one end of the capillary with a sodium silicate solution by heating the capillary with an EK 1.2 CE Capillary Burner (Electro-Kinetic Technologies, West Lothian, UK). (2) A slurry of the packing material, prepared by dispersing the OD-type packing (20 mg) in 2-propanol (1 ml) followed by sonication for several minutes, was packed into the capillary with an LC-5A HPLC pump (Shimadzu, Kyoto, Japan) by using acetonitrile as a pressurized solvent at 480 bar. (3) After completing the packing, the capillary was flushed with acetonitrile and then a 1% (v/v) sodium chloride solution at 480 bar for 1 h and 20 min, respectively. (4) A *retaining frit* was made at the point close to the temporary frit followed by making an *end frit* at an appropriate position under applying the high pressure with the sodium chloride solution. (5) The outside portion of the retaining frit was cut off, then the capillary was flushed with a mobile phase to be used at 60 bar under the reverse direction for flashing out the rest of the packing material and conditioning. (6) A detection window was made just after the end frit with the capillary burner. Finally, conditioning the capillary was carried out by flushing a mobile phase for an hour with the syringe pump.

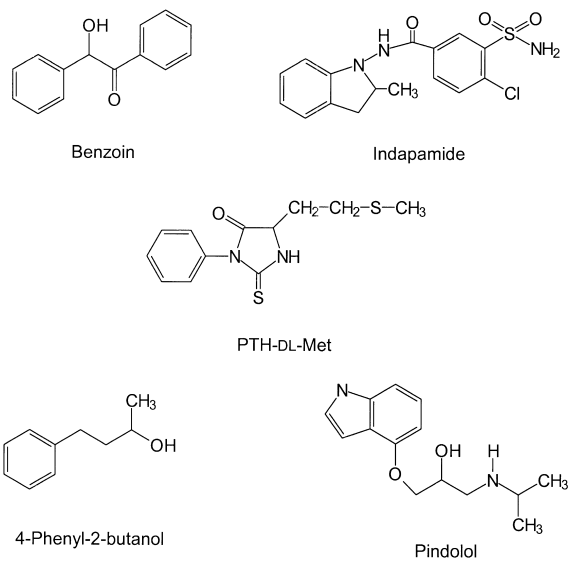

Fig. 1. Chemical structures of the enantiomers used as test solutes.

3. Results and discussion

3.1. Properties of the 3 μm OD-type packing

According to the supplier of the chiral packing, the physical properties of the 3 μm OD-type packing used in this study were the same as those of the 5 μm particle reported previously [6], i.e., the procedure of the surface treatment, the bore size of the silica-gel support, and the surface coverage of the chiral moiety, although they are not provided.

Dependences of the plate height, H, and resolution, R_s, on the EOF velocity are shown in Fig. 2a and b, respectively, where phenylthiohydantoin (PTH)-DL-Met was used as a test solute. At EOF velocity ranging from 0.2 to 1.2 mm/s (corresponding to the applied voltage of 4 and 20 kV, respectively), the almost constant and small H was obtained, where the plate height is mainly affected by mass transfer process, which is represented by the C term in van Deemter equation [19]:

$$H = A + B/u + Cu$$

where the first (A) and second (B/u) terms represent the effects of eddy diffusion and longitudinal diffusion, respectively, and u is the linear velocity. Probably the smaller value of C as well as A compared to conventional LC brought the higher column efficiency under the high EOF velocity. Under these conditions, one can expect that the higher the EOF becomes, the higher the efficiency attained. However, at an EOF of 1.4 mm/s (25 kV) H is slightly increased along with the decrease in resolution. This was possibly caused by a loss or leakage of the packings through the end frit. As for resolution, an acceptable value, $R_s > 1.5$, was obtained at an EOF velocity between ca. 0.3 and 1.2 mm/s (applied voltage 6–20 kV). These results suggest that a good and faster enantioseparation can be attained under the applied voltage of 6–20 kV.

The effect of the acetonitrile content on the retention factor, k, is shown in Fig. 3, where only 50 to 70% (v/v) acetonitrile content was employed. A linear relationship between the logarithm of the retention factor of the first migrated enantiomer, log k_1, and the acetonitrile content is observed for each enantiomeric pair, which is similar to that in conventional reversed-phase HPLC.

The effect of column temperature on the separation efficiency and resolution was investigated and the result is shown in Fig. 4. The plate height was almost independent of the column temperature, except at the lowest temperature or 15°C, while the retention factor and resolution decreased with an increase in temperature.

3.2. Enantiomer separations

The typical enantioseparations of three racemates

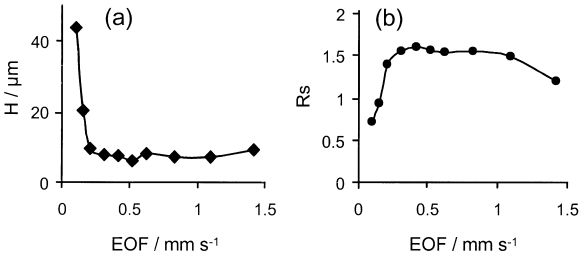

Fig. 2. Dependences of (a) the plate height (H) and (b) resolution (R_s) on the EOF velocity. Chiral packing, OD-type (3 μm); capillary, 33 cm (length of the packed portion 24 cm)×100 μm I.D.×375 μm O.D.; mobile phase composition, D (see Table 1); sample, PTH-DL-Met; injection, electrokinetic, 0.3 kV for 3 s; applied voltage, 15 kV; applied pressure, 10 bar to both inlet and outlet vials; detection wavelength, 200 nm; temperature, 25°C.

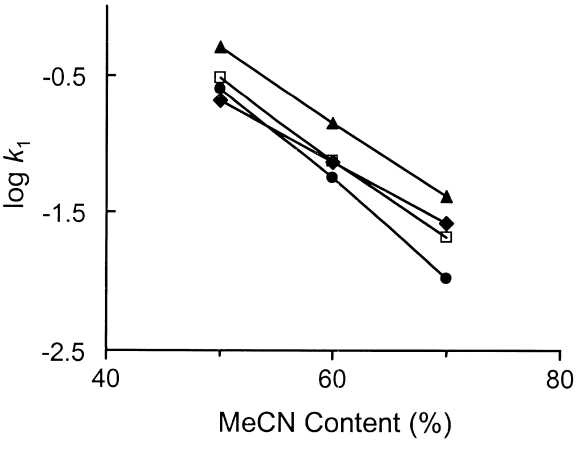

Fig. 3. Dependence of the logarithm of the retention factor of the first migrated enantiomer (log k_1) on the acetonitrile (MeCN) content. Mobile phase composition, 10 mM phosphate buffer (pH 7.2) containing acetonitrile; sample, (▲) benzoin, (□) indapamide, (●) PTH-Met, (♦) 4-phenyl-2-butanol. Other conditions as in Fig. 2.

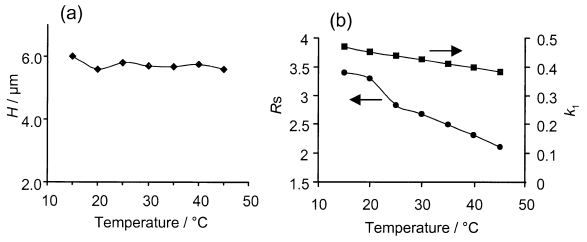

Fig. 4. The effect of the column temperature on H, R_s and k_1. Sample, racemic benzoin. Other conditions as in Fig. 2 except for temperature.

are shown in Fig. 5, where racemic (a) indapamide, (b) benzoin, and (c) PTH-Met were optically resolved. The column performance in terms of the plate number, plate height, reduced plate height, separation factor, and resolution is summarized in Table 1, in comparison with the chiral CEC system using a 5 μm OD-type previously reported [6]. Generally a higher efficiency in this 3 μm particle system was obtained compared to the 5 μm system.

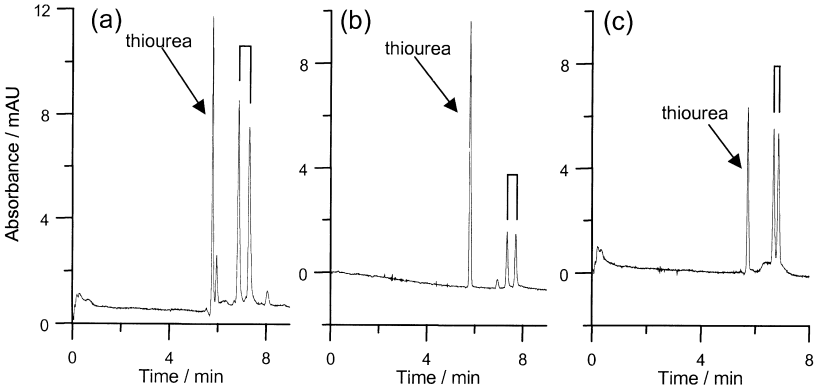

Fig. 5. Enantioseparations of racemic (a) indapamide, (b) benzoin, and (c) PTH-Met by CEC. Sample injection, electrokinetic, 3 s at (a) 0.2 kV, (b, c) 0.1 kV. Other conditions as in Fig. 2.

Table 1
Comparison of the column performance between packed with 3 and 5 μm particles

Solute	Particle diameter 5 μm[a]					Particle diameter 3 μm					
	Mobile phase[b]	α	N/m ($\cdot 10^4$)	H (μm)	h	Mobile phase[b]	R_s	α	N/m ($\cdot 10^4$)	H (μm)	h
Benzoin	B	1.17	11.1	9.0	1.8	D	2.26	1.23	21.2	4.71	1.6
			9.75	10.3	2.1				19.8	5.06	1.7
Indapamide	B	1.33	5.21	19.2	3.8	D	2.64	1.41	18.5	5.40	1.8
			4.13	24.3	4.9				15.1	6.64	2.2
PTH-DL-Met	B	1.26	3.96	25.4	5.1	D	1.29	1.19	21.6	4.63	1.5
			3.83	26.0	5.2				21.1	4.75	1.6
4-Phenyl-2-butanol	C	1.12	11.4	8.7	1.7	E	1.45	1.13	19.1	5.24	1.7
			10.8	9.2	1.8				18.7	5.35	1.8
Pindolol	A	2.32	6.88	14.6	2.9	E	9.61	2.47	10.2	9.78	3.3
			3.79	26.3	5.3				6.2	16.2	5.4

[a] Data of 5 μm packing was taken from the previous paper [6].

[b] Mobile phase compositions, A = 10 m*M* Na_2HPO_4 containing 50% (v/v) acetonitrile, B = 10 m*M* Na_2HPO_4 containing 70% (v/v) acetonitrile, C = 50 m*M* phosphate buffer (pH 4.0) containing 70% (v/v) acetonitrile, D = 10 m*M* phosphate buffer (pH 8.2) containing 80% (v/v) acetonitrile, E = 10 m*M* phosphate buffer (pH 8.2) containing 60% (v/v) acetonitrile.

Most reduced plate heights (h) observed in this system were 1.5 to 2.2, compared to 1.8 to 5.2 with the 5 μm particle. As for pindolol, large separation factor and resolution values were obtained in both 3 and 5 μm CEC systems as well as in the conventional HPLC system using a 5 μm OD-R packing [20], although the efficiency was not good.

The estimation of the repeatability of the column performance or a run-to-run ($n=5$) experiment was carried out in terms of the migration times and plate numbers using four enantiomeric pairs. The results are shown as the relative standard deviations (RSDs) in Table 2. Each RSD value for the migration time is less than 0.2%, while those for the plate number are less than 4.9%. This shows a satisfactory repeatability of this system.

The column life time was also briefly examined by using racemic 4-phenyl-2-butanol as a test solute. After 100 injections, a slightly lower plate number per unit meter (188 000) was observed compared to that in the 10th injection (222 000), as shown in Fig. 6. This implies the possibility of at least 100 repeated analyses using a single column.

Table 2
Repeatabilities of the plate number (N) and migration time ($n=5$)[a]

Solute[b]	N/m[c]		Migration time RSD (%)
	($\cdot 10^4$)	RSD (%)	
Indapamide			
1st	16.5	4.8	0.20
2nd	14.0	4.9	0.20
Benzoin			
1st	20.0	4.2	0.19
2nd	19.9	2.5	0.19
PTH-DL-Met			
1st	19.9	4.2	0.12
2nd	19.1	4.4	0.12
4-Phenyl-2-butanol			
1st	19.6	1.6	0.15
2nd	19.2	0.8	0.15

[a] Column, 240 m×100 μm I.D. packed with the OD-type (3 μm); mobile phase, D (see Table 1).
[b] 1st=the first (faster) migrated enantiomer, 2nd=the second (slower) migrated enantiomer.
[c] Data are shown as the average value and RSD.

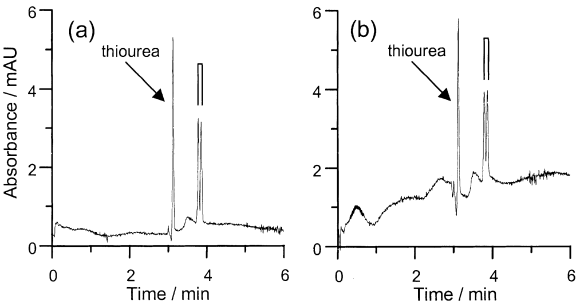

Fig. 6. Column performance test against the repeated separations: (a) at the 10th injection, (b) at the 100th injection. Sample, thiourea (first migrated peak) and 4-phenyl-2-butanol (second and third peaks). Other conditions as in Fig. 2.

3.3. Application to an optical purity test

An optical purity test was performed as an application of this chiral CEC system, employing benzoin enantiomers as model compounds. The 1.0% content of the *S*- in the *R*-forms or 4.7 μ*M S*- in 470 μ*M R*- was clearly separated and detected, as shown in Fig. 7a, and vice versa as in Fig. 7b. Similarly, the 0.1% *S*- in *R*- (470 μ*M S*- in 4700 μ*M R*-) could be separated and detected as in Fig. 7c, although the 0.1% *R*- in *S*- was not successfully detected since the first migrated peak or the *S*-form peak was tailed due to a high concentration.

The repeatability concerning the optical purity test is investigated. As for the enantioseparations of racemic benzoin at five different concentrations ranging 0.428–2.041 m*M*, a good linearity between the corrected peak area and the concentration of each enantiomer was obtained ($n=3$), resulting the regression equations along with the correlation coefficients (r) for the *S*- and *R*-forms are $y=23.3x-0.12$ ($r=0.9983$) and $y=21.0x+0.39$ ($r=0.9982$), respectively. The RSD values for the migration time, peak height, and corrected peak area for each enantiomer obtained with a 0.428 m*M* racemate as a sample are given in Table 3.

Also the RSD values are summarized in Table 3 for the case in a 1% content of one enantiomer to the other enantiomer. RSDs less than 1.4, 3.3 and 4.0% were obtained for the migration time, peak height, and corrected peak area, respectively, and these

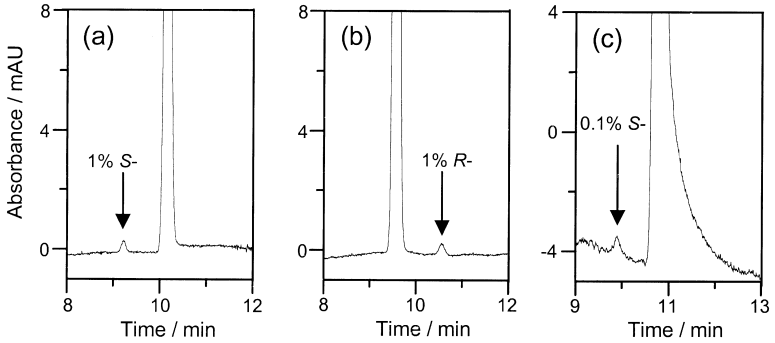

Fig. 7. Optical purity test of benzoin. Sample concentration, (a) *S*-benzoin = 4.7 μ*M*, *R*-benzoin = 470 μ*M*, (b) *S*- = 470 μ*M*, *R*- = 4.7 μ*M*, (c) *S*- = 4.7 μ*M*, *R*- = 4700 μ*M*; mobile phase composition, E (see Table 1). Other conditions as in Fig. 2.

values are acceptable for the use in the purpose of the optical purity test.

4. Conclusions

Enantiomer separations by CEC using a packed capillary with a 3 μm particle size of OD-type chiral packing have been successfully demonstrated, especially in terms of higher efficiency (typically $h = 1.5$ to 2.2) compared with the case using a 5 μm particle OD-type packing ($h = 1.8$ to 5.2). Repeatabilities in the migration time, peak height, and corrected peak area as well as the plate number in this system were acceptable for quantitative applications. A good linear relationship between the sample concentration and corrected peak area was observed. The packed capillary in this chiral CEC system can be used repeatedly more than 100 times without serious decreases in the efficiency. The application to the optical purity test was also successfully demonstrated by using benzoin enantiomers, where a 0.1% content of the first migrated enantiomer and a 1% content of the second migrated enantiomer were sufficiently separated and detected. Although the present investigation is still preliminary, the chiral CEC system should be a useful technique for chiral separations.

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Table 3
Repeatability for benzoin enantiomers

	Enantiomer and concentration					
	S-, 0.428 m*M*[a]	*R*-, 0.428 m*M*[a]	*S*-, 4.7 μ*M*	*R*-, 470 μ*M*	*S*-, 470 μ*M*	*R*-, 4.7 μ*M*
RSD (%) ($n = 3$)						
Migration time	0.40	0.43	1.36	1.34	0.04	0.06
Peak height	3.02	2.75	1.76	3.28	1.56	1.98
Peak height	3.62	3.82	1.93	3.66	2.95	3.97
A(*S*-)/*A*(*R*-)[b]			1.42		2.18	

[a] Injected as a racemate.
[b] *A*(*S*-) = peak area of the *S*-form, *A*(*R*-) = peak area of the *R*-form.

References

[1] S.C. Beale, Anal. Chem. 70 (1998) 279R.
[2] S. Li, D.K. Lloyd, Anal. Chem. 65 (1993) 3684.
[3] F. Lelièvre, C. Yan, R.N. Zare, P. Gareil, J. Chromatogr. A 723 (1996) 145.
[4] C. Wolf, P.L. Spence, W.H. Pirkle, E.M. Derrico, D.M. Cavender, G.P. Rozing, J. Chromatogr. A 782 (1997) 175.
[5] K. Krause, M. Girod, B. Chankvetadze, G. Blaschke, J. Chromatogr. A 837 (1999) 51.
[6] K. Otsuka, C. Mikami, S. Terabe, J. Chromatogr. A 887 (2000) 457.
[7] M. Girod, B. Chankvetadze, Y. Okamoto, G. Blaschke, J. Sep. Sci. 24 (2001) 27.
[8] A.S. Carter-Finch, N.W. Smith, J. Chromatogr. A 848 (1999) 375.
[9] M. Lämmerhofer, W. Lindner, J. Chromatogr. A 829 (1998) 115.
[10] L. Schweitz, L.I. Andersson, S. Nilsson, Anal. Chem. 69 (1997) 1179.
[11] S. Mayer, V. Schurig, J. High Resolut. Chromatogr. 15 (1992) 129.
[12] D.W. Armstrong, Y. Tang, T. Ward, M. Nichols, Anal. Chem. 65 (1993) 1114.
[13] O. Bruggemann, R. Freitag, M.J. Whitcombe, E.N. Vulfson, J. Chromatogr. A 781 (1997) 43.
[14] Z.J. Tan, V.T. Remcho, Electrophoresis 19 (1998) 2055.
[15] H. Hofstetter, O. Hofstetter, V. Schurig, J. Microcol. Sep. 10 (1998) 287.
[16] J.J. Pesek, M.T. Matyska, S. Menezes, J. Chromatogr. A 853 (1999) 151.
[17] Z. Liu, H. Zou, M. Ye, J. Ni, Y. Zhang, Electrophoresis 20 (1999) 2891.
[18] Z. Liu, K. Otsuka, S. Terabe, J. Sep. Sci. 24 (2001) 17.
[19] J.C. Giddings, Dynamics of Chromatography, Part 1, Marcel Dekker, New York, 1965.
[20] Application Guide for Chiral Column Selection, 2nd ed., Daicel, Tokyo, 1993, p. 39.