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Enantiomer separations by capillary electrochromatography using chiral stationary phases

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Abstract

The applicability of capillary electrochromatography (CEC) using packed capillary column to enantiomer separations was investigated. As chiral stationary phases, OD type packing materials of 5 and 3 μm particle diameters, originally designed for conventional high-performance liquid chromatography (HPLC) were employed. The chiral packing materials were packed by a pressurized method into a 100 μm I.D. fused-silica capillary. Several racemic enantiomers, such as acidic, neutral and basic drug components, were successfully resolved, typically by using acidic or basic solutions containing acetonitrile as mobile phases. The separation efficiencies for some enantiomers in the chiral CEC system using the 5 μm OD type packing were superior to those obtained in HPLC using chiral packings. The plate heights obtained for several enantiomers were 8–13 μm or the reduced plate height of 1.6–2.6, which indicates the high efficiency of this chiral CEC system.

Keywords: Chiral stationary phases, CEC; Enantiomer separation

1. Introduction

Recently, capillary electrochromatography (CEC) has become popular as a highly efficient separation techniques as well as capillary electrophoresis (CE) [1]. Since micellar electrokinetic chromatography (MEKC), where an ionic surfactant micelle is employed as a pseudostationary phase [2], is capable of separating neutral compounds as well as charged ones by using a CE instrument, sometimes CEC has been compared with MEKC especially in terms of separation performance [3]. For example, in CEC a wide range of mobile phases which contain various kinds and concentrations of organic modifiers can be used, whereas in MEKC the use of organic modifiers is normally restricted or the content of an organic modifier is limited up to 30–50% (v/v) because of the deformation of the micelle, and therefore CEC may be superior to MEKC for separations of highly hydrophobic compounds.

Enantiomer separations are one of the most important objectives in separation sciences and many reports on chiral separations by CE have appeared [4–6]. In enantioseparations by CE, several nonderivatized and derivatized cyclodextrins (CDs) and chiral surfactants are mainly used as chiral selectors added to separation solutions. In turn, only a small numbers of studies on chiral separations by CEC, however, have been reported, where several chiral moieties immobilized on silica supports [7–10] and molecularly imprinted polymers [11] were used as

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chiral stationary phases (CSPs) or a derivatized CD as a chiral additive to a separation solution [8]. Although various CSPs for high-performance liquid chromatography (HPLC) have been developed, few application studies of these CSPs in CEC have been carried out [12].

In this paper, enantiomer separations by CEC using CSPs, such as OD type packings of 5 and 3 μm particle sizes, were investigated. Since these OD type packings were originally designed for the use in HPLC, the main propose of this experiment is to find out the applicability of the CSPs to CEC. Several basic, neutral and acidic enantiomers were used as test solutes and column performance for the enantiomer separations are then discussed briefly.

2. Experimental

2.1. Materials and apparatus

Chiral packings used were OD type materials, which are silica-gel based 5 μm and 3 μm particles coated with cellulose tris(3,5-dimethylphenylcarbamate). The packings were obtained from Daicel (Tsukuba, Ibaraki, Japan). The separation capillary was a fused-silica tubing (Polymicro Technologies, Phoenix, AZ, USA) of 24 cm effective length×100 μm I.D.×375 μm O.D. packed with the chiral packing by a pressurized method. Several racemic enantiomers, the names and chemical structures given in Fig. 1, were used as test solutes. Sample

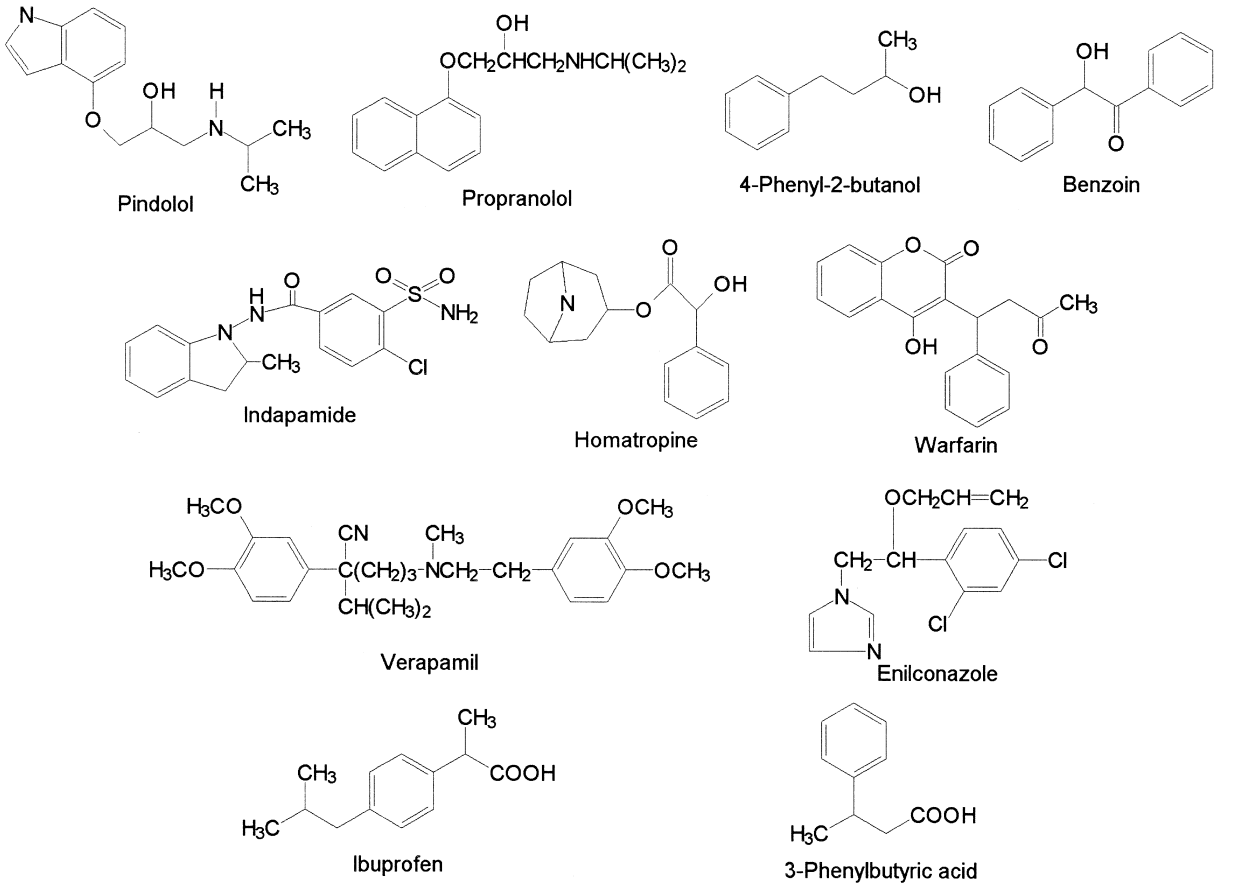

Fig. 1. Chemical structures of enantiomers used as test solutes.

Table 1
Compositions of mobile phases[a] used in this work

Abbreviation	Electrolyte solution	Acetonitrile content (%, v/v)
A	10 m*M* Na_2HPO_4	50
B	10 m*M* Na_2HPO_4	70
C	10 m*M* NaH_2PO_4	70
D	50 m*M* Phosphate buffer (pH 4.0)	70

[a] Mobile phases were prepared by mixing an electrolyte solution with an appropriate amount of acetonitrile as an organic modifier.

solutions were prepared by dissolving each enantiomer in a mobile phase at 0.1 mg/ml. Mobile phases used are summarized in Table 1. Thiourea was used as a marker of a non-retained solute or the electroosmotic flow (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.

For CEC, a Hewlett-Packard ^{3D}CE system (Waldbronn, Germany) equipped with a UV detector controlled by a ChemStation software on Windows NT was used. During a CEC separation, both inlet and outlet vials were pressurized (8 bar) to suppress the bubble formation inside the capillary. As a syringe pump, a Harvard Apparatus Model 11 (South Natick, MA, USA) was used.

2.2. Procedure for packing

The packing was carried out by a pressurized method according to Smith and Evans [13] and van den Bosch et al. [14], briefly as follows: (1) a sodium silicate solution was injected into the capillary from one end. Then a very narrow portion of the capillary outside is heated by a nichrome wire for a few seconds to form a *temporary frit*. (2) The other end of the capillary or the opposite end of the temporary frit is connected to a packer which is a stainless steel empty column of a conventional HPLC system (5 cm×4.6 mm I.D.) containing a slurry of the packing material. The slurry was prepared by dispersing the OD type packing in 2-propanol, 50 mg-packing in 1 ml 2-propanol, followed by sonication for several minutes. (3) The pressurized packing was started by using an HPLC pump, a Shimadzu LC-5A (Kyoto, Japan), operated as the constant pressure mode of ca. 420 bar and acetonitrile as a pressurized solvent. (4) After completing the packing, the pressurized solvent or acetonitrile was replaced with deionized water then pressurized again for a hour. (5) A *retaining frit* or an injection-side frit was made by heating outside of the capillary at the point close to the temporary frit under the high pressure. The frit was formed from the packing material itself. Then an *end frit* was made at an appropriate position. (6) The capillary was removed from the packer and the outside portion of the retaining frit was cut off. Then the capillary was flushed with deionized water under the reverse direction, from the retaining frit to the end frit, to wash out the rest of packing materials out side of the end frit. (7) Finally, a detection window was made just after the end frit.

3. Results and discussion

Recently, Krause et al. [15] reported on enantioseparations by nano-HPLC and CEC using polyacrylamide and polysaccharide derivatives as CSPs. As the latter CSP, silica gel of 5 μm particle size coated with cellulose tris(3,5-dimethylphenylcarbamate), which is similar to the OD type packing, was used. They obtained enantioseparations of a few chiral analytes by CEC and pressure-assisted CEC within shorter analysis time than HPLC. However, no better efficiency compared with HPLC was obtained. In the present investigation, the main purpose was to find out the applicability of CSPs which have been used in HPLC to enantiomer separations, and several basic, neutral and acidic enantiomers have been used as test solutes.

First, the performance of the OD type packing of 5 μm particle size was examined. As for basic enantio-

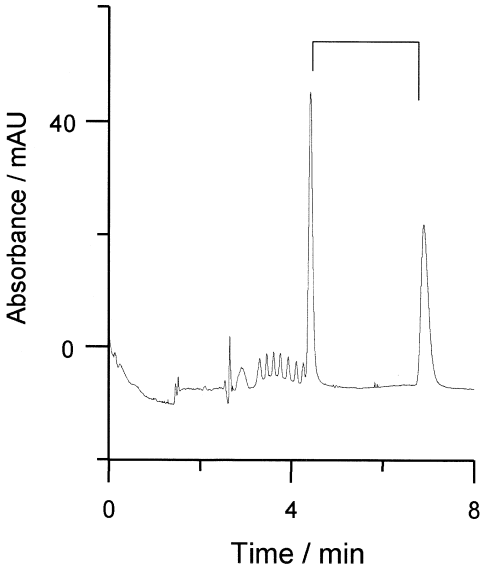

Fig. 2. Enantiomer separation of pindolol by CEC using the OD type CSP. Stationary phase, OD type; particle size, 5 μm; capillary, 33 cm (length of the packed portion 24 cm)×100 μm I.D.×375 μm O.D.; mobile phase, A (see Table 1); applied voltage, 25 kV; applied pressure, 8 bar (both inlet and outlet vials); sample injection, electrokinetic, 10 kV for 5 s; detection wavelength, 200 nm; temperature, 25°C.

mers, pindolol and propranolol were employed. Racemic pindolol was successfully resolved with the mobile phase A within 8 min, as shown in Fig. 2. Usually the enantioseparation of pindolol by reversed-phase HPLC using an OD-R packing takes longer time, e.g., 20 min [16], and therefore this implies the usefulness of CEC over conventional HPLC. In Fig. 2, small signals between t_0 (2.5 min) and the first peak (4.4 min) of pindolol enantiomers appeared accidentally probably due to a bubble formation in the capillary and/or impurities. The small signals were not reproducible, but sometimes appeared. Propranolol, however, could not be resolved completely under any conditions applied. A partial separation of two enantiomers of propranolol was observed only with the mobile phase D. Note that propranolol enantiomers can be completely separated as well as pindolol by HPLC: under a reversed-phase HPLC system using the OD-R packing, the mobile phase used was a 1.0 *M* sodium perchlorate–acetonitrile (60:40, v/v) solution. One possible reason for this discrepancy is the difference in the surface properties of stationary phases used in CEC and HPLC: although the chiral moieties are the same in both packings for CEC and HPLC, there must be some differences in properties of the silica gel supports, such as the pore size and surface coverage, but these values are not given by the supplier.

As for the enantioseparation of neutral compounds, almost complete resolution of 4-phenyl-2-butanol, benzoin and indapamide could be obtained with any mobile phases in Table 1, while homatropine and warfarin resolution was achieved with mobile phases D and B, respectively. However, partial separations were observed for verapamil and enilconazole enantiomers with all of the mobile phases in Table 1. The enantiomer separation of 4-phenyl-2-butanol with mobile phase A (50% acetonitrile) is shown in Fig. 3. Here, the retention factors for two enantiomer peaks are 0.83 and 0.94, and the average plate number is 21 000. By increasing the content of acetonitrile to 70% (v/v) or using mobile phase B, reduced retention factors 0.29 and

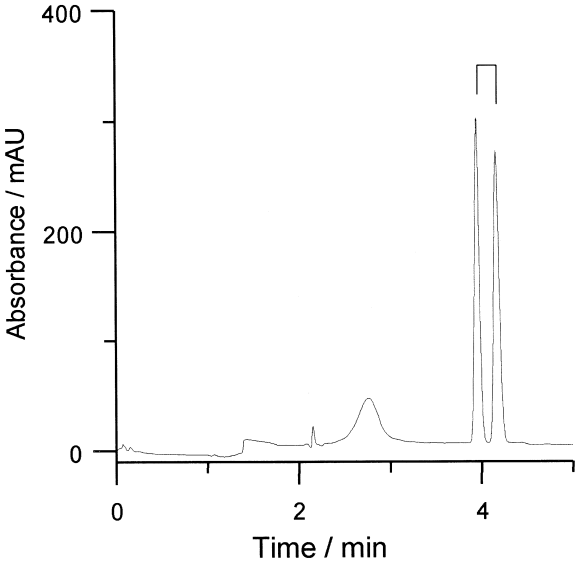

Fig. 3. Enantiomer separation of 4-phenyl-2-butanol by CEC. Conditions as in Fig. 2.

0.33 were observed, whereas the slightly reduced efficiency or the average plate number of 16 000 was observed.

The enantioseparations of benzoin with mobile phases A and B are shown in Fig. 4. Although tailed peak shapes were observed under both conditions and the plate numbers calculated cannot be compared directly, the average plate numbers are 12 000 and 21 000 in the former (50% acetonitrile) and latter (70% acetonitrile), respectively. The retention factors for two enantiomer peaks are 1.41 and 1.76 in (A) and 0.47 and 0.59 in (B).

Although the effect of the acetonitrile content on the performance has not been investigated, the retention characteristic is similar to that in reversed-phase HPLC: the higher the content of acetonitrile becomes, the smaller the retention factor appears. The velocity of the EOF in the mobile phase A (1.7 mm/s) was higher than in the mobile phase B (1.2 mm/s) under the same applied voltage, while the current in the former (28 μA) was also higher than in the latter (13 μA).

Acidic enantiomers, such as ibuprofen and 3-phenylbutyric acid, could also be resolved by using the OD type stationary phase only with mobile phase C or under acidic conditions. However, baseline fluctuations were frequently observed and efficiency was low. Under neutral or basic conditions, no enantioseparation was achieved probably due to the ionization of the solute enantiomers or an electrostatic effect.

Retention parameters and column performance for some of the test solutes are summarized in Table 2. The retention time and plate number were calculated by the HP ChemStation software. Although the column performance estimated by the plate number or plate height does not always show a good value, we can see a high efficiency in this chiral CEC system for some enantiomers, e.g., 4-phenyl-2-butanol, benzoin, homatropine and warfarin. Typically for these solutes, the plate heights and reduced plate heights are 7–13 μm and 1.4–2.5, respectively. These values are quite small and they seem impossible to achieve when a conventional HPLC system is employed: for example, the average value of plate heights and reduced plate heights for 4-phenyl-2-butanol enantiomers obtained in this CEC system are 11.4 μm and 2.3, respectively, whereas

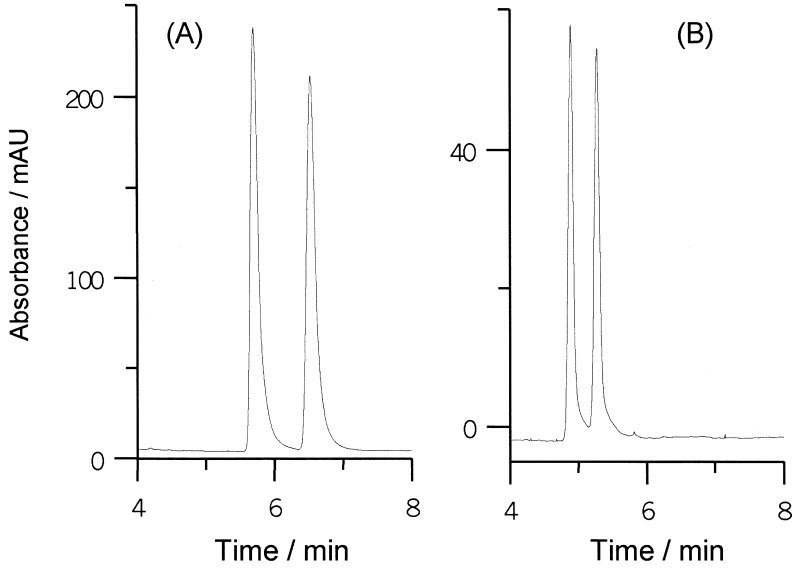

Fig. 4. Enantiomer separations of benzoin by CEC under different contents of acetonitrile. Mobile phase, 10 m*M* Na_2HPO_4–acetonitrile; acetonitrile content (v/v), (A) 50% (mobile phase A), (B) 70% (mobile phase B). Other conditions as in Fig. 2.

Table 2
Column performance[a]

Sample	Mobile Phase	t_0 (min)	t_R (min)	α[b]	N[c] ($\cdot 10^3$)	Plate height (μm)	Reduced plate height
Pindolol	A	2.53	4.41	2.32	16.5	14.6	2.9
			6.90		9.1	26.3	5.3
4-Phenyl-2-butanol	A	2.16	3.95	1.12	22.0	10.9	2.2
			4.17		20.2	11.9	2.4
Benzoin	B	3.31	4.89	1.17	21.2	11.3	2.3
			5.27		21.0	11.4	2.3
Indapamide	B	3.78	5.64	1.33	12.5	19.2	3.8
			6.25		9.9	24.3	4.9
Homatropine	D	2.33	3.18	1.19	33.9	7.1	1.4
			3.34		31.1	7.7	1.5
Warfarin	B	3.60	4.64	1.14	19.8	12.1	2.4
			4.79		18.9	12.7	2.5
Verapamil	A	2.24	9.89	1.05	9.2	26.0	5.2
			10.27		8.7	27.6	5.5
PTH-DL-Met	B	3.80	4.81	1.26	9.5	25.4	5.1
			5.07		9.2	26.0	5.2
3-Phenylbutyric acid	C	–	5.65	–	4.9	49.0	9.8
			5.86		6.2	38.7	7.7

[a] Column, 100 μm I.D., 240 mm packed with OD type (5 μm).
[b] Separation factor.
[c] Plate numbers.

values of 36 μm and 7.2, respectively, are obtained by reversed-phase HPLC using OD-R (15 cm×4.6 mm I.D.) with an aqueous solution of 30% (v/v) acetonitrile [16]. This high efficiency implies the usefulness and possibility of CEC in chiral separations, even though a large particle is employed as a stationary phase.

The use of a smaller particle, 3 μm diameter, of OD type packing was also briefly investigated. In general, we can achieve a higher efficiency by using a 3 μm packing than a 5 μm packing, if all other conditions are the same. However, good result cannot be obtained with the 3 μm particle. Although the enantiomer separation of pindolol was almost completely achieved with a 20 m*M* Na_2HPO_4 solution containing 90% (v/v) acetonitrile, the performance was inferior to that obtained with the 5 μm particle: The average plate numbers, plate height, and reduced plate height obtained with the 3 μm particle were 6000, 40 μm and 13, respectively. The reason has not been clarified yet, but may possibly be due to following two factors: (1) pressurized packing of the 3 μm particle was not successfully carried out because of an insufficient pressure during the packing procedure and (2) there are some differences in the surface properties between the 3 μm and 5 μm particles, e.g., the pore size, method of the surface treatment, surface coverage.

4. Conclusions

Enantiomer separations by CEC using CSPs have been successfully demonstrated mainly with the OD type chiral packing of 5 μm particle size. For several enantiomers, higher efficiencies and faster analysis times were achieved in this chiral CEC system than in the conventional chiral HPLC system: The retention times of enantiomers listed in Table 2 by conventional chiral HPLC with the OD-R or other chiral packings are typically longer than 11 min except for warfarin (6 min) [16]. Although the

present investigation is preliminary and the use of a smaller particle, such as 3 μm diameter or less, should be considered to achieve better efficiencies, this chiral CEC system is expected to be one of useful techniques for chiral separations. Improvements of the present method are also in progress, especially in terms of the use of smaller particles and narrower capillaries.

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References

[1] S.C. Beale, Anal. Chem. 70 (1998) 279R.
[2] K. Otsuka, S. Terabe, Bull. Chem. Soc. Jpn. 71 (1998) 2465.
[3] C. Yan, R. Dadoo, H. Zhao, R.N. Zare, Anal. Chem. 67 (1995) 2026.
[4] K. Otsuka, S. Terabe, in: N.A. Guzman (Ed.), Capillary Electrophoresis Technology, Chromatographic Science Series, No. 64, Marcel Dekker, New York, 1993, p. 617, Chapter 20.
[5] H. Nishi, J. Chromatogr. A 735 (1996) 57.
[6] R. Vespalec, P. Bocek, Electrophoresis 18 (1997) 843.
[7] S. Li, D.K. Lloyd, Anal. Chem. 65 (1993) 3684.
[8] F. Lelièvre, C. Yan, R.N. Zare, P. Gareil, J. Chromatogr. A 723 (1996) 145.
[9] C. Wolf, P.L. Spence, W.H. Pirkle, E.M. Derrico, D.M. Cavender, G.P. Rozing, J. Chromatogr. A 782 (1997) 175.
[10] M. Lämmerhofer, W. Lindner, J. Chromatogr. A 829 (1998) 115.
[11] L. Schweitz, L.I. Andersson, S. Nilsson, Anal. Chem. 69 (1997) 1179.
[12] S.J. Grieb, A. Maxwell, J.P. Foley, Presented at the 19th International Symposium on Capillary Chromatography and Electrophoresis, Wintergreen, VA, May 1997.
[13] N.W. Smith, M.B. Evans, Chromatographia 38 (1994) 649.
[14] S.E. van den Bosch, S. Heemstra, J.C. Kraak, H. Poppe, J. Chromatogr. A 755 (1996) 165.
[15] K. Krause, M. Girod, B. Chankvetadze, G. Blaschke, J. Chromatogr. A 837 (1999) 51.
[16] Application Guide for Chiral Column Selection, 2nd Edition, Daicel Chemical, Tokyo, 1993, p. 39.