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Enantiomeric separations of cationic and neutral compounds by capillary electrochromatography with monolithic chiral stationary phases of β-cyclodextrin-bonded negatively charged polyacrylamide gels

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Abstract

Enantiomeric separation by capillary electrochromatography with β-cyclodextrin-bonded negatively charged polyacrylamide gels was examined. The columns used are capillaries filled with a negatively charged polyacrylamide gel, a so-called monolithic stationary phase, to which allyl carbamoylated β-CD (AC-β-CD) derivatives covalently bind. The capillary wall is activated first with a bifunctional reagent to make the resulting gel bind covalently to the inner surface of the fused-silica tubing. Enantiomeric separations of 15 cationic compounds were achieved using the above-mentioned columns and mobile phases of 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 7.0 or 9.0) or 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 7.0) containing an achiral crown ether (18-crown-6). Enantiomeric separations of two neutral compounds were also achieved using 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 9.0) as a mobile phase. High efficiencies of up to 150 000 plates m^{-1} were obtained. Both the within- and between-run reproducibilities of retention time and separation factor were good. The reproducibilities of retention time and separation factor for three different columns prepared from a different batch of monomers were acceptable. The gel-filled capillaries were stable for at least 3 months with intermittent use, utilizing the mobile phase of 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 9.0).

Keywords: Enantiomer separation; Chiral stationary phases, electrochromatography; Cationic compounds; Neutral compounds; Allyl carbamoylated β-cyclodextrin derivatives

1. Introduction

Enantiomeric separation has always been important in various fields, such as natural product research, stereospecific synthesis, chiral drugs in the pharmaceutical industry and chiral compounds in environmental fields. Especially in the pharmaceutical industry, a large number of drugs which have one or more asymmetric centers exist as racemates. Since the pharmacological activity and metabolism of two enantiomers of a certain drug often differ, analytical methods which can allow enantiomeric separation in a short time, at low cost, with high efficiency, with good resolution and with good reproducibility are required for chiral purity control, pharmacokinetic studies and other work. Capillary

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electrophoresis (CE) has recently received much attention for the enantiomeric separations and recently considered as a complementary technique to high-performance liquid chromatography (HPLC) and gas chromatography. Some of its typical features are relatively short analysis time, high efficiency, easy exchange of separation media and minimal sample volume requirement in comparison with HPLC. Numerous studies of enantiomeric separations of various compounds including drugs by CE have been reported and summarized [1,2].

Capillary electrochromatography (CEC), which combines the desirable features of both HPLC and CE, has become increasingly popular. CEC is a useful separation method which affords higher theoretical plate number and superior efficiency due to its pluglike flow profile, compared with micro-HPLC which uses the same packing material. In CEC, as in HPLC, the solutes of a given sample mixture can be preferentially separated based on differences in distribution ratios between the mobile phase and the stationary phase. Compounds having a charge can also be influenced by the applied voltage, leading to differential migration caused by electrophoresis, as in CE. Therefore both charged and uncharged compounds can be separated according to their differential migration through the column based on the solute's interaction between the two phases or a combination of such interactions and the inherent electrophoretic mobilities of the solutes [3]. So far, capillaries packed with the typical stationary phase for HPLC were used in various CEC studies [3,4]. While, at present monolithic stationary phases, which are ungranular polymeric separation media, are increasingly attracting attention in the field of CEC due to their simple preparation method, wide variety of functionalization and good stability [5–15].

Thus far, enantiomeric separation by CEC has been performed by the following methods:

(1) Capillaries packed with chiral stationary phases (CSPs) for HPLC: enantiomeric separations have been achieved using α_1-acid glycoprotein [16], β-cyclodextrins (β-CDs) and their derivatives [17–19], human serum albumin [20], (*S*)-naproxen-derived or (3*R*,4*S*)-Whelk-O [21], quinine-based anion exchange type [22], polysaccharide derivatives [23] and antibiotics [24,25] as the stationary phases.

(2) Capillaries with the inner surface coated with CSPs: enantiomeric separations of a number of non-steroidal anti-inflammatory drugs and mephobarbitol have been achieved using capillaries with the inner surface coated with a chiral dimethylpolysiloxane consisting of covalently linked permethyl-β-CD [26–30]. Likewise, the separation of epinephrine enantiomers was reported with a γ-CD-coated capillary [31]. Neat cellulose derivatives were also employed as solid non-immobilized CSPs [32].

(3) Capillaries filled (packed column) or coated (open-tubular) with molecularly imprinted polymer as monolithic CSPs for CEC: the technique for preparing polymeric media having specific molecular recognition for the template utilized [33–38] is interesting from the viewpoint of the separation principle. However, the electrochromatograms obtained occasionally show broad peaks and an unstable baseline.

Very recently, enantiomeric separations with monolithic stationary phases for CEC (not molecularly imprinted polymer) were reported [39]. We have also reported enantiomeric separations of various compounds by CEC with monolithic CSPs [40–42] by reference to the concept of enantiomeric separation by capillary gel electrophoresis [43].

This paper reports the successful enantiomeric separations of various cationic and neutral compounds by CEC using β-CD-bonded negatively charged polyacrylamide gel-filled capillaries. The reproducibilities and stability of the columns are also presented.

2. Experimental

2.1. Apparatus

CEC experiments were carried out at room temperature (approximately 25°C) with a Jasco CE-800 system, which consisted of a ±30 kV high-voltage power supply (890-CE) and a UV–VIS detector (870-CE) (Japan Spectroscopic, Tokyo, Japan). A Shimadzu C-R4AX (Kyoto, Japan) instrument was used for data collection and manipulation. Capillary temperature was not controlled during the experiments. Separations were performed with fused-silica tubing (CElect-UVT, Supelco, Bellefonte, PA, USA) of 75 μm I.D.×363 μm O.D. filled with a negatively

charged polyacrylamide gel. Each analyte shown in Fig. 1 was dissolved in a solution with the same composition as the mobile phase or a solution mixed with the same volume of dimethyl sulfoxide (DMSO), and was introduced into the anodic end of the columns by electrokinetic injection. The detection wavelength was 240, 245 or 254 nm, depending on the absorbance spectrum of each analyte.

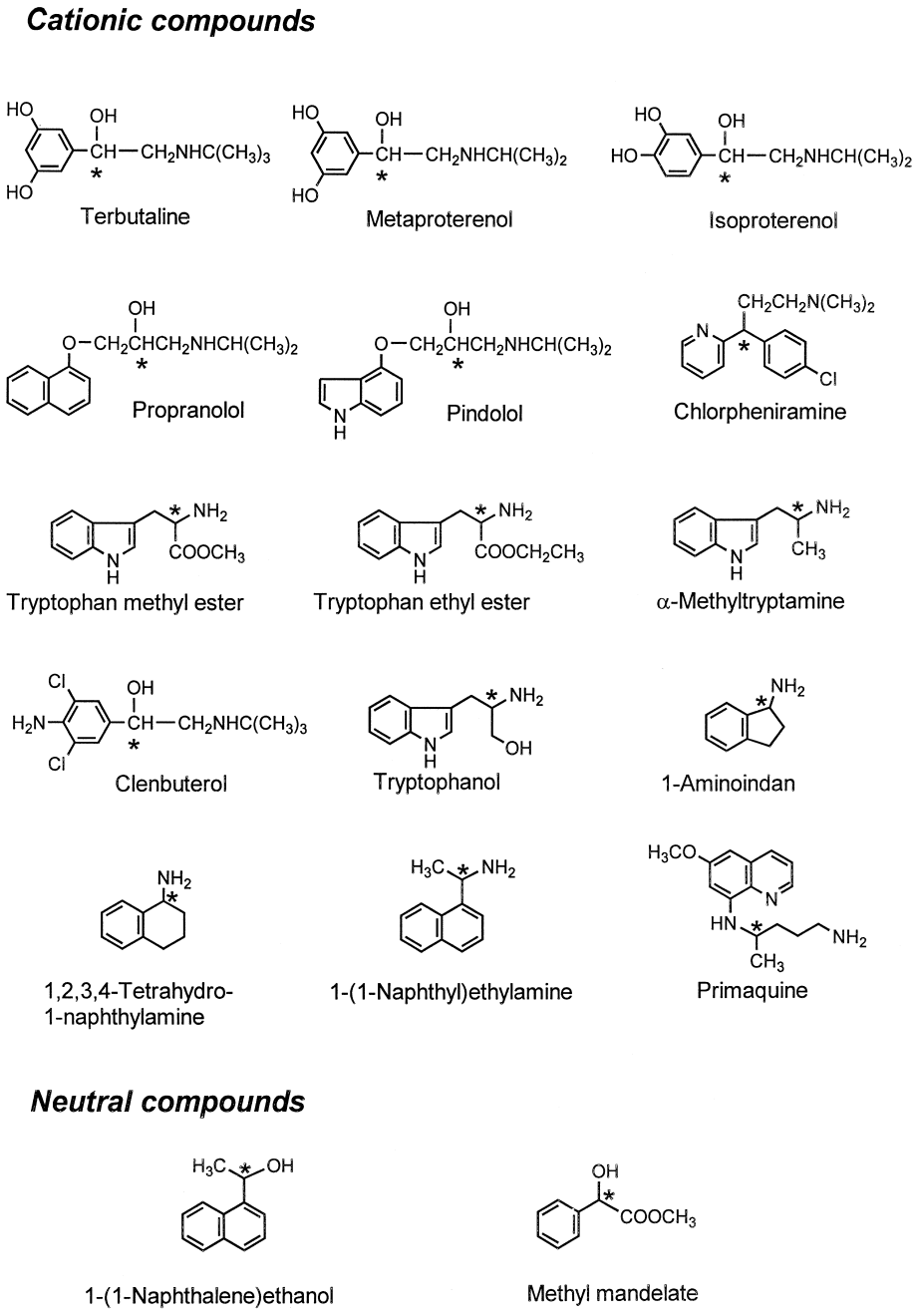

Fig. 1. Chemical structures of the separated cationic and neutral compounds.

2.2. Chemicals

N,*N*′-Methylenebisacrylamide (BIS), *N*,*N*,*N*′,*N*′-tetramethylethylendiamine (TEMED), ammonium peroxodisulfate (APS), di-phosphorous pentaoxide, β-CD, DL-isoproterenol, benzoin, DMSO, acetic acid and distilled water were purchased from Nacalai Tesque (Kyoto, Japan). Methacrylic acid 3-trimethoxysilylpropyl ester, 2-acrylamido-2-methylpropanesulfonic acid (AMPS), DL-tryptophan ethyl ester hydrochloride, chlorpheniramine maleate, 1,2-diphenylethanol, 1-(1-naphthalene)ethanol, DL-mandelic acid methyl ester, DL-1-(1-naphthyl)ethylamine, (*R*)-(+)-1-(1-naphthyl)ethylamine, (*S*)-(−)-1-(1-naphthyl)ethylamine, 1-aminoindan and 18-crown-6 were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Acrylamide (AA), boric acid, terbutaline sulfate, DL-propranolol hydrochloride and pindolol were from Wako (Osaka, Japan). Tris-(hydroxymethyl)aminomethane (Tris), metaproterenol, DL-tryptophan methyl ester hydrochloride, DL-tryptophanol oxalate salt, clenbuterol hydrochloride and primaquine diphosphate salt were from Sigma (St. Louis, MO, USA). Allylisocyanate, α-methyltryptamine and 1,2,3,4-tetrahydro-1-naphthylamine hydrochloride were purchased from Aldrich (Milwaukee, WI, USA). Dehydrated pyridine was obtained from Kanto Kagaku (Tokyo, Japan).

2.3. Synthesis of allyl carbamoylated β-CD (AC-β-CD)

AC-β-CD was synthesized according to a general method [44,45] with some modifications: (1) a 5-g portion of β-CD was dried in vacuo at 60°C for 8 h in the presence of di-phosphorous pentaoxide; (2) the dried β-CD was dissolved in 70 ml of dry pyridine; (3) 1 ml of allylisocyanate was added dropwise to the β-CD pyridine solution with stirring; (4) the reaction solution was heated at 80°C and kept standing for 6 h under nitrogen atmosphere; (5) the reaction solution was cooled down to room temperature; (6) after pyridine had been evaporated in vacuo, the raw product was purified by repeated recrystallization from acetone.

2.4. Preparation of columns

The capillary columns for enantiomeric separations were prepared according to the method described in Ref. [5], with some modifications as follows. The inner surface of the capillaries was treated with a reagent mixture consisting of 40 μl of methacrylic acid 3-trimethoxsilylpropyl ester mixed with 10 ml of 6 mmol l^{-1} acetic acid at room temperature for more than 3 h. After the modified capillary had been rinsed thoroughly with distilled water, the capillary was filled with a mixture of various concentrations of AC-β-CD, AA, BIS and AMPS in a 100 mmol l^{-1} Tris–150 mmol l^{-1} boric acid buffer (pH 8.1) containing 0.5–1.0 mg ml^{-1} APS and 3 μl ml^{-1} TEMED with suction under reduced pressure. After the capillary had been filled, both ends were dipped into vials filled with the polymerization solution, and the capillary was left standing for more than 5 h. Finally, the capillary was set in the CE instrument and preelectrophoresed about 6 h until the baseline of the detector output became stable.

The nomenclature introduced by Hjertén [46] was used to represent the total acrylamide concentration (%T) and the degree of cross-linking (%C):

$$\%T = 100\,(a + b + c)/V$$

$$\%C = 100\,b/(a + b + c)$$

where a, b and c are the mass of AA, BIS and AMPS (in g), respectively, and V is the volume (in ml). In addition to these values, the mole percentage of AMPS, defined by Fujimoto et al. [6], was used:

$$\%S = 100\,\gamma/(\alpha + \beta + \gamma)$$

where α, β and γ are the molarities of AA, BIS and AMPS, respectively.

2.5. Calculation

In the following discussion, the separation factor (α) was calculated for convenience by

$$\alpha = t_2/t_1$$

where t_1 is the retention time of the first eluted enantiomer and t_2 is the retention time of the antipode [47], because with charged analytes it is not possible to obtain an accurate value of μ_0, which is the mobility that the analyte would have in the column with no chromatographic partitioning onto

the stationary phase [16]. The resolution (R_s) was calculated by:

$$R_s = 2\,(t_2 - t_1)/(w_1 + w_2)$$

where w_1 is the bottom peak width of the first eluted enantiomer and w_2 is the bottom peak width of the antipode [47].

3. Results and discussion

3.1. Gel characteristics

According to the procedures described in the Experimental section, AC-β-CD-bonded negatively charged polyacrylamide gel-filled capillaries, in which electroosmotic flow (EOF) toward the cathode would be generated, were prepared. The proposed schematic structure of the gels is shown in Fig. 2. The cross-linked polyacrylamide gels bind covalently to the inner surface due to pretreatment with methacrylic acid 3-trimethoxsilylpropyl ester, and AMPS and AC-β-CD are attached to the gels. The sulfo group derived from AMPS is ionized in solutions over a wide range of pH and permits the generation of EOF which flows toward the cathode. Accordingly, electrophoretic migration of cationic compounds and the EOF proceed in the same direction. The β-CD structure derived from AC-β-CD works as a chiral selector. The formation of AC-β-CD was supported by the absorption maxima of 1706 cm^{-1} in the infrared spectrum. A positive ion electrospray ionization mass spectrum of the product revealed the presence of AC-β-CD having 2–5 degrees of substitution.

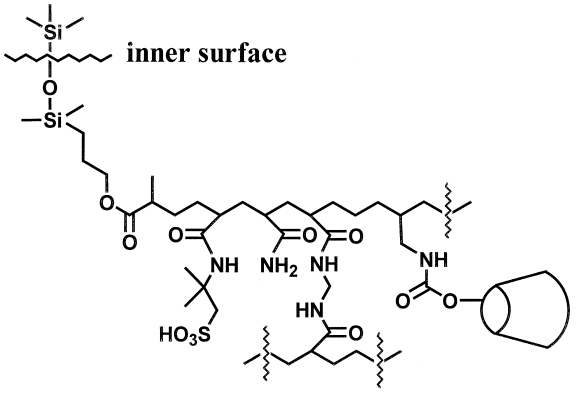

Fig. 2. Proposed schematic structures of AC-β-CD-bonded negatively charged polyacrylamide gels.

When a phosphate or a borate buffer was used as the mobile phase, high current, heat generation and bubble formation were observed. On the other hand, low conductivity buffers such as Tris–boric acid buffer would be preferred for the AC-β-CD-bonded negatively charged polyacrylamide gel-filled capillaries. Therefore, Tris–boric acid buffer solutions were usually used as the mobile phase throughout this study. The dependence of the EOF velocities of an AC-β-CD-bonded negatively charged polyacrylamide gel-filled capillary on the pH of the mobile phase was determined using DMSO as an unretained solute marker at an applied voltage of 270 V cm^{-1}. Fig. 3 shows that the EOF velocity increases with increasing pH, reaching a maximum at a pH of around 8. However, the enantiomeric separations in this study were examined using 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 7.0, 8.1 and 9.0), which bore no relation to this result.

3.2. Enantiomeric separation of cationic compounds

Enantiomeric separations of various cationic com-

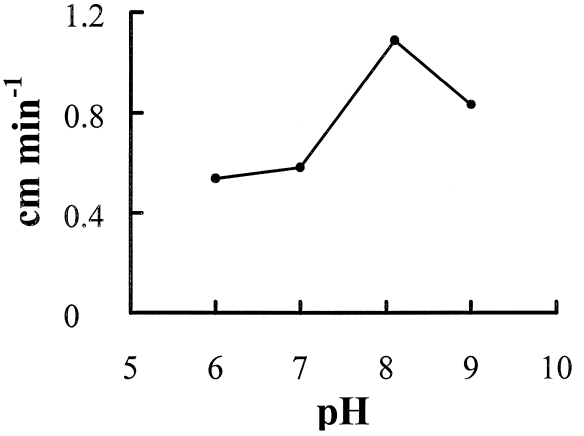

Fig. 3. Effect of pH value of buffers used for the mobile phase on electroosmotic flow velocity. Conditions: capillary, fused-silica capillary [37 cm (effective length 20 cm)×75 μm I.D.); %*T*, 5; %*C*, 5; %*S*, 5.5; AC-β-CD, 50 mg ml^{-1}; mobile phase, 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer; unretained solute marker, dimethyl sulfoxide; applied voltage, 270 V cm^{-1}; detection wavelength, 210 nm.

pounds were investigated using AC-β-CD-bonded negatively charged polyacrylamide gel-filled capillaries. As a result of the analyses, enantiomeric separations of six cationic compounds (terbutaline, metaproterenol, isoproterenol, propranolol, pindolol, chlorpheniramine) were achieved using the mobile phase of 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 9.0). Baseline separations were obtained for terbutaline, metaproterenol and propranolol. These six compounds were also resolved enantiomerically with 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 8.1), however lower efficiencies were obtained (data not shown). Good enantiomeric separations of four cationic compounds (tryptophan methyl ester, tryptophan ethyl ester, α-methyltryptamine, clenbuterol) were achieved using 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 7.0). The results are summarized in Table 1. Typical electrochromatograms of metaproterenol and clenbuterol are shown in Fig. 4. The EOF velocities were 1.8 and 1.1 cm min^{-1} when terbutaline and metaproterenol were injected, respectively, using DMSO as an unretained solute marker.

3.3. Enantiomeric separation of neutral compounds

Neutral compounds without electrophoretic mobility should be transported through the columns by EOF and detected. Enantiomeric separations of neutral compounds using AC-β-CD-bonded gel-filled capillaries were also investigated using 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 9.0). We obtained enantiomeric separations of two neutral compounds as summarized in Table 1, and the electrochromatogram of 1-(1-naphthalene)ethanol is shown in Fig. 5. The EOF velocities was 1.3 cm min^{-1} when 1-(1-naphthalene)ethanol was injected, using DMSO as an unretained solute marker.

Acidic compounds such as dansyl amino acids could not be detected within 90 min throughout this study, probably due to their counter-electroosmotic migration.

Table 1
Enantiomeric separation of cationic and neutral compounds

Compound	Retention time (min)		Plate number (m^{-1})		α	R_s	AC-β-CD (mg ml^{-1})	Mobile phase[c]
	t_1	t_2	N_1	N_2				
Cationic compounds								
Terbutaline[a]	31.4	33.8	106 000	87 000	1.08	2.49	50	1
Metaproterenol[a]	25.4	26.9	58 000	48 000	1.06	1.87	80	1
Isoproterenol[a]	21.6	22.2	33 000	28 000	1.03	0.68	100	1
Propranolol[a]	39.7	41.1	144 000	121 000	1.04	1.90	80	1
Pindolol[a]	47.7	49.4	41 000	25 000	1.04	0.93	50	1
Chlorpheniramine[a]	41.5	47.1	3000	2000	1.14	0.94	10	1
Tryptophan methyl ester[a]	27.4	28.5	42 000	24 000	1.04	0.96	50	2
Tryptophan ethyl ester[a]	37.1	40.2	34 000	27 000	1.09	1.77	50	2
α-Methyltryptamine[a]	38.7	40.4	111 000	86 000	1.04	1.75	50	2
Clenbuterol[b]	29.0	32.7	74 000	51 000	1.13	2.86	50	2
Neutral compounds								
1-(1-Naphthalene)ethanol[a]	89.5	94.9	44 000	32 000	1.06 (1.09[d])	1.57	80	1
Methyl mandelate[a]	40.5	41.6	11 000	6000	1.03 (1.07[d])	<0.5	100	1

[a] Conditions: capillary, fused-silica capillary [60–70 cm (effective length 35 cm)×75 μm I.D.]; %*T*, 5 or 7; %*C*, 5 or 10; %*S*, 5.5, 5.6 or 6; applied voltage (current), 171–357 V cm^{-1} (8–28 μA); detection wavelength, 240, 245 or 254 nm; sample concentration, 1–10 mmol l^{-1}; sample injection, electrokinetic method (220 kV, 13 s).

[b] Conditions: capillary, fused-silica capillary [37 cm (effective length 20 cm)×75 μm I.D.]; %*T*, 5; %*C*, 5; %*S*, 5.5; applied voltage (current), 324 V cm^{-1} (10 μA); detection wavelength, 254 nm; sample concentration, 2 mmol l^{-1}; sample injection, electrokinetic method (5 kV, 2 s).

[c] 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 9.0) (1), 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 7.0) (2).

[d] $\alpha=(t_2-t_0)/(t_1-t_0)$: where t_0 is the retention time of dimethyl sulfoxide.

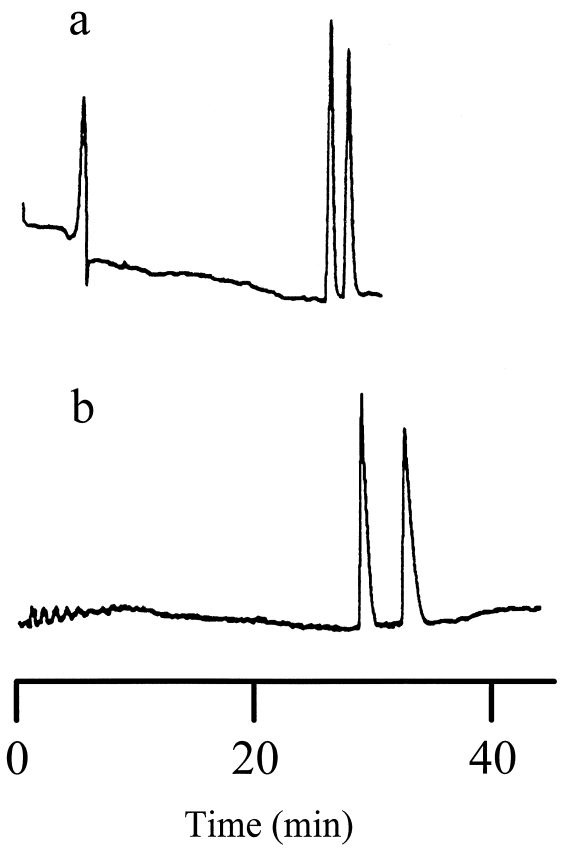

Fig. 4. Enantiomeric separation of metaproterenol (a) and clenbuterol (b) with AC-β-CD-bonded negatively charged polyacrylamide gel-filled capillaries. Conditions: (a) capillary, fused-silica capillary [70 cm (effective length 35 cm)×75 μm I.D.]; %*T*, 5; %*C*, 10; %*S*, 5.6; applied voltage (current), 270 V cm^{-1} (11 μA); detection wavelength, 240 nm; sample concentration, 5 mmol l^{-1}; sample injection, electrokinetic method (18 kV, 3 s); other conditions as in Table 1; (b) conditions as in Table 1.

3.4. Enantiomeric separation of primary amino compounds

Recently, it was reported that the addition of an achiral crown ether (18-crown-6) for cyclodextrin-based separation in CE could lead to or promote enantiomeric separations of primary amino compounds when the enantiomers could not be separated or showed poor resolution with cyclodextrins alone [48–50]. Our CEC study with AC-β-CD-bonded gel-filled capillaries yielded poor or no resolutions of tryptophanol, 1-aminoindan, 1,2,3,4-tetrahydro-1-naphthylamine, 1-(1-naphthyl)ethylamine and primaquine enantiomers. The enantiomeric separations of these five compounds were investigated using a mobile phase of 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 7.0) containing the 18-crown-6. The results of the analyses are shown in Table 2. The addition of 10 mmol l^{-1} 18-crown-6 to the mobile phase did not affect the enantiomeric separation of tryptophanol. On the other hand, enhancements of the enantiomeric separations of 1-aminoindan, 1,2,3,4-tetrahydro-1-naphthylamine, 1-(1-naphthyl)ethylamine and primaquine were observed with increasing retention time. A typical electrochromatogram of 1-(1-naphthyl)ethylamine is shown in Fig. 6. The separation mechanism seems to be the same as that in CE described in Refs. [48–50]. The primary amino group is protonated and forms a host–guest complex with the 18-crown-6 in the mobile phase. The hydrophobic portion of the host–guest complex is incorporated into the cavity of the β-CD attached to the gels. Finally, the primary amino compound is sandwiched between the 18-crown-6 and the β-CD as proposed in Fig. 7 and thus the enantioselective recognition is enhanced.

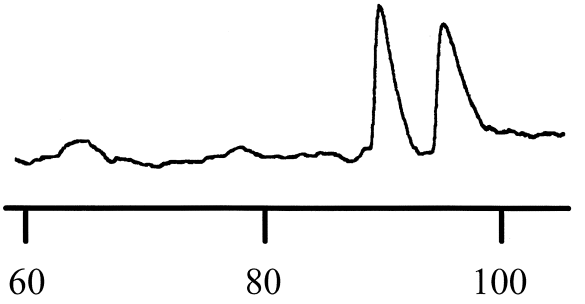

Fig. 5. Enantiomeric separation of 1-(1-naphthalene)ethanol with an AC-β-CD-bonded negatively charged polyacrylamide gel-filled capillary. Conditions: capillary, fused-silica capillary [70 cm (effective length 35 cm)×75 μm I.D.]; %*T*, 5; %*C*, 10; %*S*, 5.6; applied voltage (current), 267 V cm^{-1} (13 μA); detection wavelength, 240 nm; sample concentration, 3 mmol l^{-1}; sample injection, electrokinetic method (20 kV, 2 s). Other conditions as in Table 1.

3.5. Reproducibility and stability

Reproducibility is a concern in analytical techniques. The within-run reproducibilities of retention time and separation factor were examined for ter-

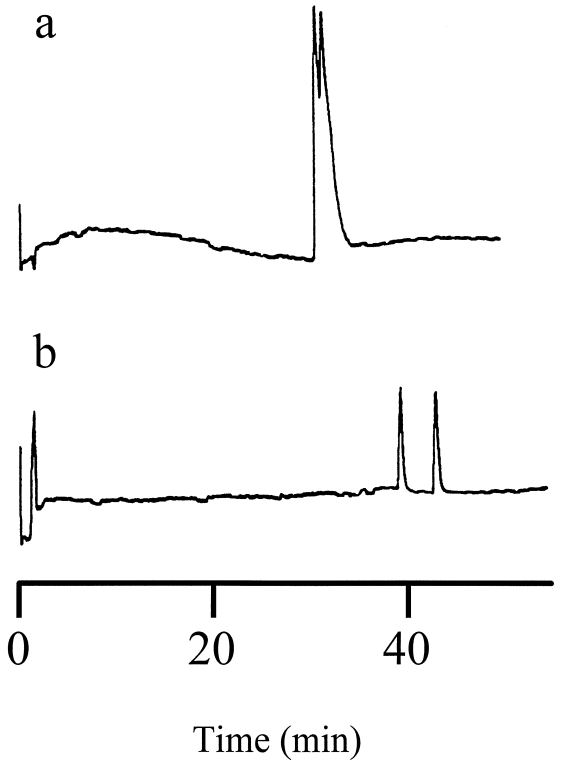

Fig. 6. Enantiomeric separation of 1-(1-naphthyl)ethylamine with an AC-β-CD-bonded negatively charged polyacrylamide gel-filled capillary. (a) Electrochromatogram using 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 7.0) as a mobile phase. (b) Electrochromatogram using 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 7.0) containing 10 mmol l^{-1} of 18-crown-6. Conditions: sample concentration, 9 mmol l^{-1}. Other conditions as in Table 2.

butaline and metaproterenol. The between-run reproducibilities of retention time and separation factor were examined for terbutaline and metaproterenol. The relative standard deviations (RSDs) of the within- and between-run reproducibilities of retention time were less than 1.2 and 3.3% over the six injections, respectively. Those of the separation factor were 0.2 and less than 0.3%, respectively. The reproducible results obtained are summarized in Table 3. Shown in Table 4 are the same parameters for three different columns prepared from a different batch of monomers for enantiomeric separations of terbutaline and metaproterenol. Some variation can be seen between the three columns with regard to the retention time. Separation factors were reproducible in spite of the fact that no temperature controller was used during the polymerization process.

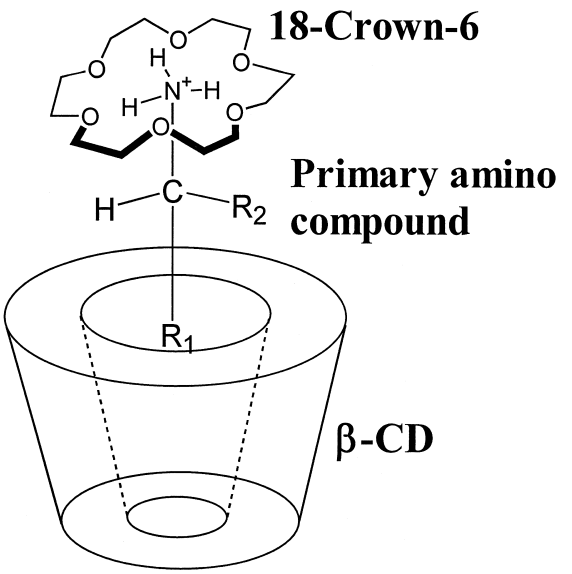

Fig. 7. Schematic molecular model of the complex consisting of 18-crown-6, primary amino compound and β-CD.

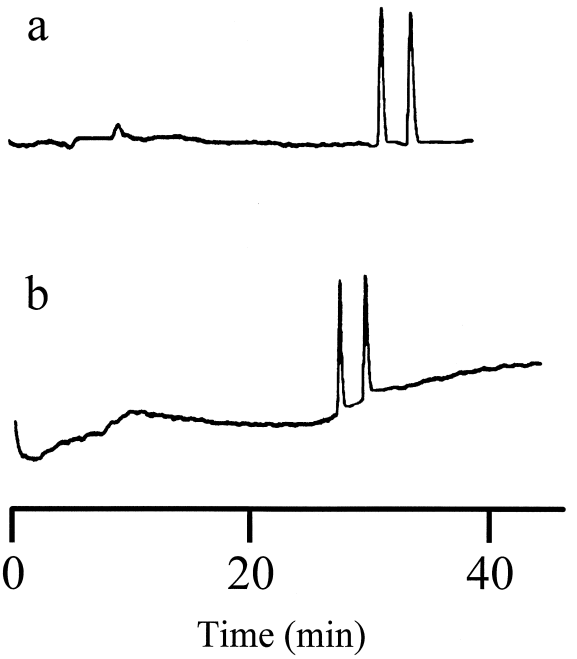

Fig. 8. Stability of an AC-β-CD-bonded negatively charged polyacrylamide gel-filled capillary. (a) Electrochromatogram of terbutaline obtained immediately after preparation of the column, (b) after intermittent use for 3 months. Conditions: capillary, fused-silica capillary [70 cm (effective length 35 cm)×75 μm I.D.]; %*T*, 5; %*C*, 5; %*S*, 5.5; AC-β-CD, 50 mg ml^{-1}; applied voltage (current), 214 V cm^{-1} (10 μA); detection wavelength, 240 nm. Other conditions as in Table 1.

Table 2
Enantiomeric separation of primary amino compounds

Compound	Retention time (min)		Plate number (m^{-1})		α	R_s	Mobile phase[c]
	t_1	t_2	N_1	N_2			
Tryptophanol[a]	28.5	29.2	18 000	11 000	1.02	<0.5	1
	33.7	34.6	11 000	7000	1.03	<0.5	3
1-Aminoindan[a]	14.2	14.7	30 000	15 000	1.04	0.77	1
	18.5	20.6	7000	3000	1.12	0.97	2
1,2,3,4-Tetrahydro-1-naphthylamine[a]	16.1	16.8	13 000	8000	1.04	0.64	1
	18.0	19.7	14 000	5000	1.09	1.01	2
1-(1-Naphthyl)ethylamine[a]	30.1	30.8	16 000	9000	1.02	<0.5	1
	39.0	42.6	140 000	150 000	1.09	4.84	3
Primaquine[b]	34.9	–	38 000	–	1.00	0	1
	57.4	60.7	70 000	53 000	1.06	1.45	3

[a] Conditions: capillary, fused-silica capillary [62 cm (effective length 35 cm)×75 μm I.D.]; %*T*, 5; %*C*, 5; %*S*, 5.5; concentration of AC-β-CD, 50 mg ml^{-1}; applied voltage (current), 242 V cm^{-1} (10 μA); detection wavelength, 254 nm; sample concentration, 1–10 mmol l^{-1}; sample injection, electrokinetic method (15 kV, 2 s).

[b] Conditions: capillary, fused-silica capillary [37 cm (effective length 20 cm)×75 μm I.D.]; %*T*, 5; %*C*, 5; %*S*, 5.5; concentration of AC-β-CD, 50 mg ml^{-1}; applied voltage (current), 270 V cm^{-1} (8 μA); detection wavelength, 254 nm; sample concentration, 1 mmol l^{-1}; sample injection, electrokinetic method (1 kV, 2 s).

[c] 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 7.0) (1), 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 7.0) containing 5 mmol l^{-1} of 18-crown-6 (2), 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 7.0) containing 10 mmol l^{-1} of 18-crown-6 (3).

The stability of the columns prepared was also examined. The electrochromatogram of terbutaline obtained immediately after preparation of the column was compared with that after its intermittent use for 3 months as shown in Fig. 8, using the mobile phase of 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 9.0). Some variation was seen between the two electrochromatograms with regard to the retention times, with one reason for the variation being a difference in the batch of the mobile phase. The separation factors were reproducible [(a) $\alpha = 1.08$, (b) $\alpha = 1.08$], so the prepared columns seem stable for intermittent use for at least 3 months.

3.6. Effect of AC-β-CD concentration on parameters

Fig. 9 shows the dependence of retention time of the first eluted enantiomer (t_1), α and R_s of terbutaline, metaproterenol and propranolol on four AC-β-CD concentrations. For all the studied compounds, t_1 increased together with an increase in the

Table 3
Within- and between-run reproducibilities of retention time and separation factor[a]

Compound	Within-run (RSD, %, $n=6$[b])			Between-run (RSD, %, $n=6$[b])		
	t_1	t_2	α	t_1	t_2	α
Terbutaline	1.2	1.2	0.2	3.2	3.3	0.2
Metaproterenol	0.5	0.4	0.1	3.0	3.1	0.3

[a] Conditions: capillary, fused-silica capillary [70 cm (effective length 35 cm)×75 μm I.D.]; %*T*, 5; %*C*, 10; %*S*, 5.6; AC-β-CD, 50 mg ml^{-1}; applied voltage (current), 286 V cm^{-1} (13 μA); detection wavelength, 240 nm. Other conditions as in Table 1.

[b] n is the total number of measurements carried out.

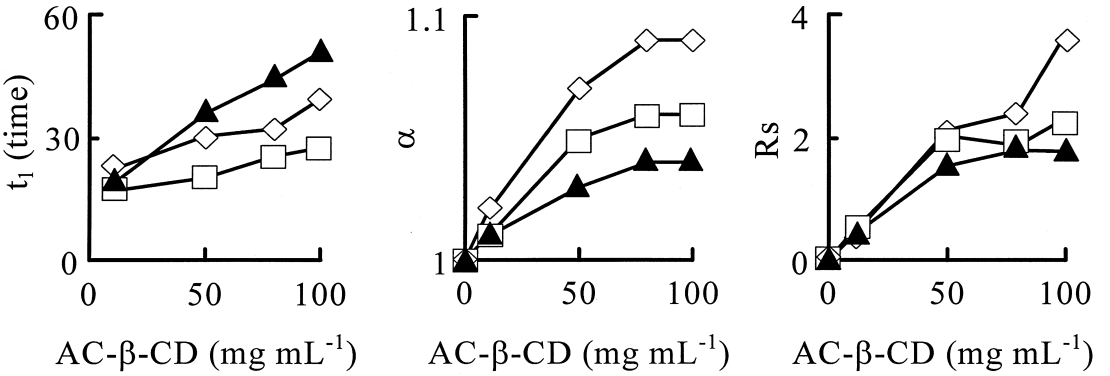

Fig. 9. Effect of AC-β-CD concentration on (a) retention time of the first eluted enantiomer (t_1), (b) separation factor (α) and (c) resolution (R_s) of terbutaline (◇), metaproterenol (□) and propranolol (▲). Conditions: capillary, fused-silica capillary (effective length 35 cm×75 μm I.D.); %*T*, 5; %*C*, 10; %*S*, 5.6; applied voltage, 214 V cm^{-1} (terbutaline), 257 V cm^{-1} (metaproterenol and propranolol); detection wavelength, 240 nm. Other conditions as in Table 1.

Table 4
Performance of three different columns[a]

Column No.	Terbutaline (average, $n=6$[b])			Metaproterenol (average, $n=6$[b])		
	t_1 (min)	t_2 (min)	α	t_1 (min)	t_2 (min)	α
1	32.6	36.0	1.10	27.9	29.7	1.06
2	29.2	32.2	1.10	26.5	28.2	1.07
3	32.4	35.8	1.10	27.5	29.3	1.07

[a] Conditions as in Table 3.
[b] n is the total number of measurements carried out.

AC-β-CD added to the polymerization solution (Fig. 9a). This led us to suppose that AC-β-CD was immobilized due to the higher concentration in the polymerization solution. An increase of α was recorded using higher concentrations of AC-β-CD in the range 0–80 mg ml^{-1}, and the value of α almost reached a plateau using concentrations of AC-β-CD in more than 80 mg ml^{-1} for all the compounds studied (Fig. 9b). R_s kept increasing with increasing concentrations of AC-β-CD in the range 0–100 mg ml^{-1} for terbutaline. An increase of R_s was recorded using higher concentrations of AC-β-CD in the range 0–50 mg ml^{-1}, and the value of R_s almost reached a plateau using concentrations of AC-β-CD in more than 50 mg ml^{-1} for metaproterenol and propranolol (Fig. 9c). Therefore, α and R_s in enantiomeric separations could be controlled by changing the AC-β-CD concentration added to the polymerization solution.

4. Conclusion

This paper has described a simple and reliable method for enantiomeric separation by CEC. The enantiomeric separations of various cationic and neutral compounds were achieved using capillaries filled with AC-β-CD-bonded negatively charged polyacrylamide gels, which need no frits to keep the stationary phase in place, and 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 7.0 or 9.0) or 200 mmol l^{-1} Tris–300 mmol l^{-1} boric acid buffer (pH 7.0) containing the 18-crown-6 as a mobile phase. High efficiencies were obtained for some cationic compounds. Good within- and between-run reproducibilities of retention time and separation factor were obtained. The same parameters for three different columns prepared from a different batch of monomers were also compared. Some variations of the retention times were observed among the three

columns. Separation factors were reproducible in spite of the fact that no temperature controller was used during the polymerization process. The gel-filled capillaries prepared were stable to intermittent use for at least 3 months.

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