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Enantiomeric separations of primary amino compounds by capillary electrochromatography with monolithic chiral stationary phases of chiral crown ether-bonded negatively charged polyacrylamide gels

Takashi Koide*, Kyoji Ueno

Shionogi Research Laboratories, Shionogi & Co., Ltd., 12-4 Sagisu 5-Chome, Fukushima-ku, Osaka 553-0002, Japan

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Abstract

A novel enantiomeric separation method by capillary electrochromatography with chiral crown ether-bonded negatively charged polyacrylamide gels is presented. Two kinds of chiral crown ether derivatives, (+)-tetraallyl 18-crown-6 carboxylate and (+)-18-crown-6 tetracarboxylic acid 2-allyl ester were synthesized and allowed to covalently bind to a negatively charged polyacrylamide gel, a so-called monolithic stationary phase, respectively. The gel was placed in fused-silica tubing, the walls of which had been activated with a bifunctional reagent to make the resulting gel bind covalently to the inner surface. Enantiomeric separations of 12 primary amino compounds were achieved using these columns and mobile phases of 200 m*M* triethanolamine–300 m*M* boric acid buffers with high efficiencies of up to 135 000 plates m^{-1}. 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 13 months with intermittent use for 3 months followed by storage at room temperature for 10 months. The result of the optical purity test of alanine-2-naphthylamide is also described.

Keywords: Enantiomer separation; Electrochromatography; Chiral stationary phases, electrochromatography; Monolithic stationary phases; Polyacrylamide gels; Crown ethers; Amines; Amides

1. Introduction

Enantiomeric separation is important in various fields, such as natural product research, stereospecific synthesis, chiral drugs in the pharmaceutical industry and chiral compounds in environmental studies. In the pharmaceutical industry, it is important because 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 some drugs often differ, analytical methods which can allow enantiomeric separation in a short time, at low cost, with high efficiency, and with good resolution and reproducibility are required for chiral purity control, pharmacokinetic studies and other work. Recently, capillary electrophoresis (CE) has been receiving much attention for enantiomeric separations and is considered to be a complementary technique to high-performance liquid chromatography (HPLC) and gas chromatography. Some of its

*Corresponding author. Tel.: +81-6-6458-5861; fax: +81-6-6458-0987.

E-mail address: takashi.koide@shionogi.co.jp (T. Koide).

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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 powerful 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 possessing a charge can also be affected by the applied voltage, leading to differential migration caused by electrophoresis, as in CE. Therefore both charged and uncharged species 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]. Capillaries packed with a typical stationary phase for HPLC have been used in various CEC studies [3,4], while monolithic stationary phases, which are ungranular polymeric separation media, are increasingly attracting attention for CEC because of their simple preparation method, wide variety of functionalization and good stability [5–15].

Thus far, enantiomeric separation by CEC has been performed with various columns such as capillaries packed with chiral stationary phases (CSPs) for HPLC [16–21], capillaries with the inner surface coated with CSPs [22–24], capillaries filled with molecularly imprinted polymer as monolithic CSPs for CEC [25,26] and capillaries filled with monolithic CSPs for CEC (not molecularly imprinted polymer) [27–29]. We have also reported enantiomeric separation methods of CEC with monolithic CSPs immobilizing β-CDs as chiral selectors [30–34] with reference to the concept of enantiomeric separation by capillary gel electrophoresis [35].

The macrocyclic polyethers having oxygen atoms as donor atoms are generally called crown ethers. One of the fundamental characteristics of crown ethers is selective complexation ability. They bind the cationic portion of alkali and alkaline earth salts and that of ammonium salts into their cavity. The formation of host–guest complexes is based on ion–dipole interactions between the cation and the donor oxygen atoms in the cyclic polyether [36]. (+)-18-Crown-6 tetracarboxylic acid (18C6H4) shown in Fig. 1a, which is one of chiral crown ethers, was synthesized for the first time by Behr et al. [37] and used as buffer additive in CE by Kuhn and coworkers [38,39] for the enantiomeric separations of racemic amino acids. Recently, 18C6H4 seems to be the first choice for enantiomeric separations of primary amino compounds in CE in spite of its high cost [40,41].

In this paper, we report the successful enantiomeric separations of primary amino compounds by CEC with monolithic chiral stationary phases of 18C6H4 derivative-bonded negatively charged polyacrylamide gels. The reproducibilities and stability of the columns are also presented.

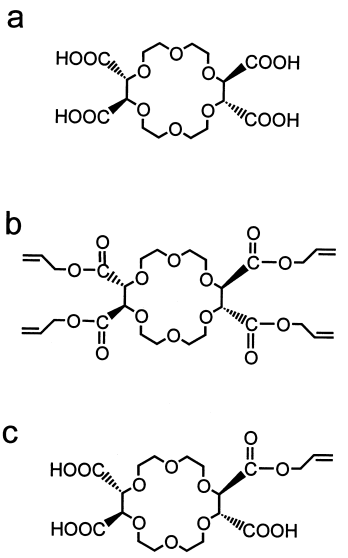

Fig. 1. Chemical structures of (+)-18-crown-6 tetracarboxylic acid (a), (+)-tetraallyl 18-crown-6 carboxylate (b) and (+)-18-crown-6 tetracarboxylic acid 2-allyl ester (c).

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 C-R4AX integrator (Shimadzu, Kyoto, Japan) 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 chiral crown ether-bonded negatively charged polyacrylamide gel. Each analyte shown in Fig. 2 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 sample solution for the optical purity test was prepared by spiking ca. 0.2% DL-alanine-2-naphthylamide to L-alanine-2-naphthylamide solution. The detection wavelength was 254 nm.

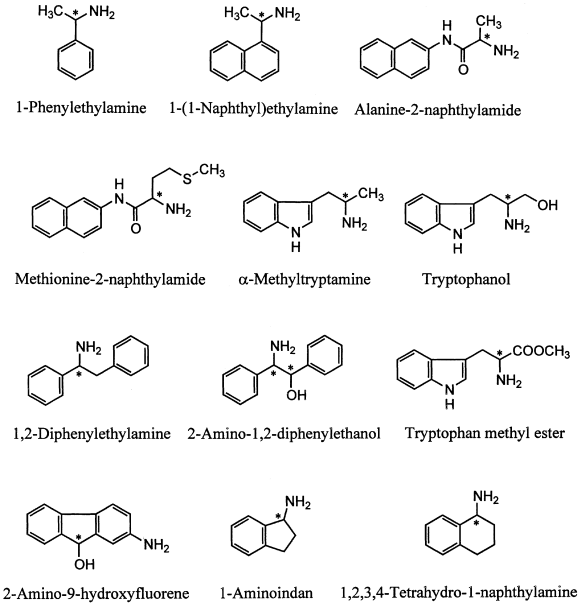

Fig. 2. Chemical structures of the separated primary amino compounds.

The HPLC system, which consisted of an L-7100 pump (Hitachi, Tokyo, Japan), an SPD-10AV UV–Vis detector (Shimadzu), a C-R4AX integrator (Shimadzu) and a CMA/200 autosampler (BAS, Tokyo, Japan), was used for the preparative HPLC of (+)-18-crown-6 tetracarboxylic acid 2-allyl ester (Fig. 1c). The guard column was an Inertsil ODS (10 μm, 50×20 mm I.D.) and the preparative column was an Inertsil ODS (10 μm, 250×20 mm I.D.). Both of the columns were purchased from GL Sciences (Tokyo, Japan) and operated at room temperature (approximately 25°C). The detection wavelength was 220 nm.

2.2. Chemicals

N,*N*′-Methylenebisacrylamide (Bis), *N*,*N*,*N*′,*N*′-tetramethylethylendiamine (TEMED), ammonium peroxodisulfate (APS), DMSO, acetic acid, distilled water and 0.1 *M* sodium hydroxide solution were purchased from Nacalai Tesque (Kyoto, Japan). Methacrylic acid 3-trimethoxysilylpropyl ester, 2-acrylamido-2-methylpropanesulfonic acid (AMPS), 1,2-diphenylethanol, DL-1-(1-naphthyl)ethylamine, (*R*)-(+)-1-(1-naphthyl)ethylamine, (*S*)-(−)-1-(1-naphthyl)ethylamine, (*R*)-(+)-1-phenylethylamine, (*S*)-(−)-1-phenylethylamine, 1-aminoindan, (1*R*,2*S*)-(−)-2-amino-1,2-diphenylethanol and *N*,*N*-diisopropylethylamine were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Acrylamide (AA), boric acid, 2,2′,2″-nitrilotriethanol, allyl bromide and trifluoroacetic acid (TFA) were from Wako (Osaka, Japan).

L-Alanine-β-naphthylamide, DL-methionine-β-naphthylamide, DL-tryptophanol oxalate salt and DL-tryptophan methyl ester hydrochloride were from Sigma (St. Louis, MO, USA). DL-Alanine-2-naphthylamide, α-methyltryptamine, 1,2,3,4-tetrahydro-1-naphthylamine hydrochloride, 2-amino-9-hydroxyfluorene, (1*S*,2*R*)-(+)-2-amino-1,2-diphenylethanol and 18C6H4 were purchased from Aldrich (Milwaukee, WI, USA). Acetonitrile for HPLC and potassium chloride were obtained from Kanto Kagaku (Tokyo, Japan). *N*,*N*-Dimethylformamide (DMF) and diethyl ether were from Katayama Kagaku (Osaka, Japan).

2.3. Synthesis of (+)-tetraallyl 18-crown-6 carboxylate

(+)-Tetraallyl 18-crown-6 carboxylate (Fig. 1b) was synthesized according to the following method: (1) a 50-mg portion of 18C6H4 was dissolved in 1.5 ml of DMF; (2) 100 μl of allyl bromide (10 equivalents) and 116 μl of *N*,*N*-diisopropylethylamine (6 equivalents) was added to the 18C6H4 DMF solution with stirring; (3) the reaction solution was kept standing for about 12 h at room temperature (approximately 25°C); (4) after DMF and excess allyl bromide and *N*,*N*-diisopropylethylamine had been evaporated in vacuo, the raw product was dissolved in 2 ml of diethyl ether; (5) after *N*,*N*-diisopropylethylammonium salt had been removed by filtration under reduced pressure, diethyl ether was evaporated in vacuo. (+)-Tetraallyl 18-crown-6 carboxylate (colorless oil) was obtained in 87% total yield.

$[\alpha]_D^{25}$; +75.9° (c=1.0, CH_3OH). IR (KBr); 3084 ($CH=CH_2$), 1747 (C=O), 1648 ($CH=CH_2$) cm^{-1}. Mass spectrometry (MS) m/z; 601 $[M+H]^+$. High-resolution (HR) MS m/z calculated for $C_{28}H_{41}O_{14}$ $[M+H]^+$: 601.2496, found: 601.2489. ^{1}H-nuclear magnetic resonance (NMR) (C^2HCl_3) δ; 3.74, 3.69 (8H, m, O–C$\underline{H}_2$–CH_2–O–CH–COO), 3.76, 3.87 (8H, m, O–CH_2–C$\underline{H}_2$–O–CH–COO), 4.42 (4H, s, O–C$\underline{H}$–COO), 4.65 (8H, m, COO–C$\underline{H}_2$–CH=CH_2), 5.25 (4H, dd, J=10.2, 1.4 Hz, COO–CH_2–CH=C$\underline{H}_2$ *trans*), 5.35 (4H, dd, J=17.2, 1.4 Hz, COO–CH_2–CH=C$\underline{H}_2$ *cis*). ^{13}C-NMR (C^2HCl_3) δ; 65.80 (t, COO–$\underline{C}H_2$–CH=CH_2), 70.42 (t, O–$\underline{C}H_2$–CH_2–O–CH–COO), 71.41 (t, O–CH_2–$\underline{C}H_2$–O–CH–COO), 80.04 (d, O–$\underline{C}$H–COO), 118.66 (t, COO–CH_2–CH=$\underline{C}H_2$), 131.63 (d, COO–CH_2–$\underline{C}$H=CH_2), 169.01 (s, CH–$\underline{C}$OO–CH_2–CH=CH_2).

2.4. Synthesis of (+)-18-crown-6 tetracarboxylic acid 2-allyl ester

(+)-18-Crown-6 tetracarboxylic acid 2-allyl ester was synthesized according to the following method: (1) a 200-mg portion of 18C6H4 was dissolved in 3 ml of DMF; (2) 59 μl of allyl bromide (1.5 equivalents) and 93 μl of *N*,*N*-diisopropylethylamine (1.2 equivalents) was added to the 18C6H4 DMF solution with stirring; (3) the reaction solution was kept standing for about 12 h at room temperature; (4) after DMF and excess allyl bromide and *N*,*N*-diisopropylethylamine had been evaporated in vacuo, the raw product was dissolved in 200 μl of a mixture of acetonitrile–water (10:90, v/v) for injection into the preparative HPLC system; (5) after the preparative HPLC followed by evaporation of the mobile phase in vacuo, (+)-18-crown-6 tetracarboxylic acid 2-allyl ester (white powder) was obtained.

The preparative HPLC conditions were as follows: a mixture of 0.1% (v/v) aqueous TFA solution–acetonitrile (70:30, v/v) was used as the mobile phase (flow-rate 9.5 ml min^{-1}) for the preparative HPLC. The guard column was an Inertsil ODS (10 μm, 50×20 mm I.D.) and the preparative column was an Inertsil ODS (10 μm, 250×20 mm I.D.). As (+)-18-crown-6 tetracarboxylic acid 2-allyl ester was eluted and detected at about 16 min, the eluate around the time of 16 min was collected.

$[\alpha]_D^{25}$; +63.5° (c=1.0CH_3OH). MS m/z; 481 $[M+H]^+$, 503 $[M+Na]^+$. HR-MS m/z calculated for $C_{19}H_{28}O_{14}Na$ $[M+Na]^+$: 503.1376, found: 503.1392. ^{1}H-NMR ($C^2H_3SOC^2H_3$) δ; 3.55 (8H, m, O–C$\underline{H}_2$–CH_2–O–CH–COO), 3.55, 3.72 (8H, m, O–CH_2–C$\underline{H}_2$–O–CH–COO), 4.19 (2H, s, O–C$\underline{H}$–COOH), 4.22 (1H, d, J=5.0 Hz, O–C$\underline{H}$–COOH), 4.34 (1H, d, J=5.0 Hz, O–C$\underline{H}$–COO–CH_2–CH=CH_2), 4.62 (2H, m, COO–C$\underline{H}_2$–CH=CH_2), 5.24 (1H, dd, J=10.6, 1.5 Hz, COO–CH_2–CH=C$\underline{H}_2$ *trans*), 5.38 (1H, dd, J=17.1, 1.5 Hz, COO–CH_2–CH=C$\underline{H}_2$ *cis*), 12.95 (3H, bs, O–CH–COO$\underline{H}$). ^{13}C-NMR ($C^2H_3SOC^2H_3$) δ; 64.95 (t, COO–$\underline{C}H_2$–CH=CH_2), 69.26 (t, O–$\underline{C}H_2$–CH_2–O–CH–COO), 69.85, 69.94 (t, O–CH_2–$\underline{C}H_2$–O–CH–COOH), 69.94 (t, O–CH_2–$\underline{C}H_2$–O–CH–COO–CH_2–CH=C$\underline{H}_2$), 79.23 (d, O–$\underline{C}$H–COO), 117.97 (t, COO–CH_2–CH=$\underline{C}H_2$), 132.08 (d, COO–CH_2–$\underline{C}$H=CH_2), 168.69 (s, CH–$\underline{C}$OO–CH_2–CH=CH_2), 170.35, 170.50, 170.53 (s, CH–$\underline{C}$OOH).

2.5. Preparation of columns

The capillary columns for enantiomeric separations were prepared according to the method described in Ref. [6], 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 m*M* 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 (+)-tetraallyl 18-crown-6 carboxylate, KCl, AA, Bis and AMPS in a 100 m*M* Tris–150 m*M* boric acid buffer (pH 8.1) or a mixture of (+)-18-crown-6 tetracarboxylic acid 2-allyl ester, AA, Bis and AMPS in a 100 m*M* Tris–150 m*M* boric acid buffer (pH 8.1) containing 0.5–1.0 mg ml^{-1} APS and 3 μl ml^{-1} TEMED from one end to the other end 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 for about 8 h until the baseline of the detector output became stable.

The nomenclature introduced by Hjertén [42] and Fujimoto [6] was used to represent the total acrylamide concentration (%T), the degree of cross-linking (%C) and the mole percentage of AMPS (%S):

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

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

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

where a, b and c are the masses of AA, Bis and AMPS (in g), respectively, V is the volume (in ml), and α, β and γ are the molarities of AA, Bis and AMPS, respectively.

2.6. Calculation

The separation factor (α) was calculated for convenience with:

$$\alpha = t_2/t_1$$

where t_1 is the retention time of the first eluted enantiomer and t_2 is the retention time of the antipode [39], 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 a column with no chromatographic partitioning onto the stationary phase [16]. The resolution (R_s) was calculated with:

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

where w_1 is the bottom peak width of the first eluted enantiomer and w_2 is the bottom peak width of the antipode [39].

3. Results and discussion

3.1. Gel characteristics

Chiral crown ether-bonded negatively charged polyacrylamide gel-filled capillaries, in which electroosmotic flow (EOF) toward the cathode would be generated, were prepared as described in the Experimental section. The proposed schematic structures of the gels are shown in Fig. 3. The cross-linked polyacrylamide gels bind covalently to the inner surface due to pretreatment with methacrylic acid 3-trimethoxsilylpropyl ester, and AMPS and chiral crown ether 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 primary amino compounds and the EOF proceed in the same direction.

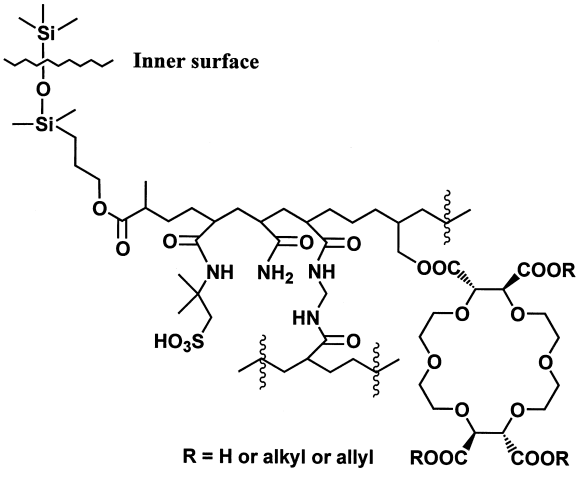

Fig. 3. Proposed schematic structures of chiral crown ether-bonded negatively charged polyacrylamide gels.

3.2. (+)-Tetraallyl 18-crown-6 carboxylate-bonded negatively charged polyacrylamide gel-filled capillary

3.2.1. Enantiomeric separation of primary amino compounds

Low-conductivity buffers such as triethanolamine–boric acid buffer are preferred for the charged polyacrylamide gel-filled capillaries, according to the information in Refs. [31,33]. Therefore, triethanolamine–boric acid buffer solutions, boric acid solution, and a mixture of acetonitrile and those solutions were usually used as the mobile phase throughout this study.

Enantiomeric separations of various primary amino compounds were investigated using (+)-tetraallyl 18-crown-6 carboxylate-bonded negatively charged polyacrylamide gel-filled capillaries. As a result of the analyses, baseline enantiomeric separations of three primary amino compounds [1-(1-naphthyl)ethylamine, α-methyltryptamine, 1,2-diphenylethylamine] were achieved using a mixture of 300 m*M* aqueous boric acid solution–acetonitrile (50:50, v/v) as a mobile phase. Enantiomeric separations of seven compounds (1-phenylethylamine, alanine-2-naphthylamide, methionine-2-naphthylamide, 1-aminoindan, tryptophanol, 2-amino-1,2-diphenylethanol, tryptophan methyl ester) were achieved using 200 m*M* triethanolamine–300 m*M* boric acid buffer (pH 6.0) or a mixture of the buffer–acetonitrile (80:20, v/v). The results are summarized in Table 1. High efficiencies of more than 100 000 plates m^{-1} were obtained for alanine-2-naphthylamide and α-methyltryptamine. Typical electrochromatograms of 1-(1-naphthyl)ethylamine, alanine-2-naphthylamide and tryptophanol are shown in Fig. 4. The EOF velocity was 7 cm min^{-1} when DMSO as an unretained solute marker was analyzed at an applied voltage of +125 V cm^{-1} using the mobile phase of 200 m*M* triethanolamine–300 m*M* boric acid buffer (pH 6.0).

3.2.2. Reproducibility and stability

Reproducibility is a concern with analytical techniques. The within-run reproducibilities of retention time and separation factor were examined for 1-(1-naphthyl)ethylamine, alanine-2-naphthylamide and tryptophanol. The between-run reproducibilities of retention time and separation factor were examined for 1-(1-naphthyl)ethylamine and tryptophanol. The relative standard deviations (RSDs) of the within- and between-run reproducibilities of retention time were less than 2.9 and 2.1% over the six injections, respectively. Those of the separation factor were less than 0.9 and 0.5%, respectively. The reproducible results obtained are summarized in Table 2. Shown in Table 3 are the same parameters for three different columns prepared from a different batch of mono-

Table 1
Enantiomeric separation of primary amino compounds with (+)-tetraallyl 18-crown-6 carboxylate-bonded negatively charged polyacrylamide gels[a]

Compound	Retention time (min)		Plate number (m^{-1})		α	R_s	Chiral selector (m*M*)	Mobile phase[b]	Applied voltage (V cm^{-1})	Current (μA)
	t_1	t_2	N_1	N_2						
1-(1-Naphthyl)ethylamine	29.2	30.3	79 000	85 000	1.04	1.36	40	2	125	1
1-Phenylethylamine	23.2	25.1	19 000	31 000	1.08	1.14	20	1	147	5
Alanine-2-naphthylamide	26.1	31.3	135 000	51 000	1.20	7.17	20	3	188	6
Methionine-2-naphthylamide	35.5	40.3	64 000	39 000	1.14	3.60	20	3	188	6
α-Methyltryptamine	34.6	35.7	105 000	109 000	1.03	1.49	40	2	125	1
1-Aminoindan	25.3	26.4	6000	3000	1.04	<0.5	20	1	125	6
1,2-Diphenylethylamine	33.4	36.6	78 000	85 000	1.10	3.75	40	2	125	1
Tryptophanol	31.6	32.9	46 000	24 000	1.04	1.00	20	1	125	5
2-Amino-1,2-diphenylethanol	39.0	40.5	7000	2000	1.04	<0.5	20	1	100	5
Tryptophan methyl ester	34.5	35.4	18 000	12 000	1.03	<0.5	20	1	125	7

[a] Conditions: capillary, fused-silica capillary [80 cm (effective length 35 cm)×75 μm I.D.]; %T, 5.0; %C, 5.0; %S 5.5; detection wavelength, 254 nm; sample concentration, 1–10 m*M*; sample injection, electrokinetic method.

[b] 1=200 m*M* triethanolamine–300 m*M* boric acid buffer (pH 6.0), 2=300 m*M* aqueous boric acid solution–acetonitrile (50:50, v/v), 3=[200 m*M* triethanolamine–300 m*M* boric acid buffer (pH 6.0)]–acetonitrile (80:20, v/v).

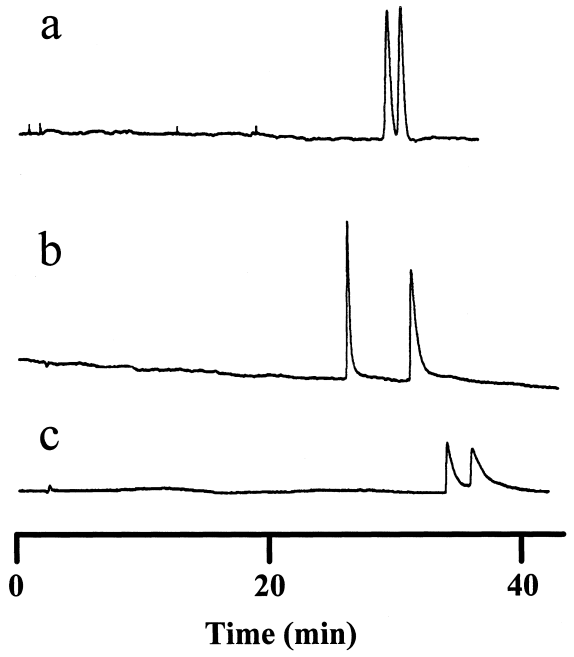

Fig. 4. Enantiomeric separation of 1-(1-naphthyl)ethylamine (a), alanine-2-naphthylamide (b) and tryptophanol (c) with (+)-tetraallyl 18-crown-6 carboxylate-bonded negatively charged polyacrylamide gel-filled capillaries. Conditions as in Table 1.

mers for enantiomeric separations of alanine-2-naphthylamide and tryptophanol. Some variation can be seen between the three columns with respect to the retention time. Separation factors were reproducible in spite of the fact that no temperature controller was used during the polymerization process.

The stability of the columns prepared was also examined. The electrochromatogram of alanine-2-naphthylamide obtained immediately after preparation of the column was compared with that after its intermittent use for 3 months followed by storage at room temperature for 10 months as shown in Fig. 5. During the storage, both ends of the capillaries were dipped into vials filled with 200 m*M* triethanolamine–300 m*M* boric acid buffer (pH 6.0). The prepared columns seem stable for at least 13 months judging from the electrochromatograms of Fig. 5.

3.2.3. Optical purity test

The CEC method with (+)-tetraallyl 18-crown-6 carboxylate-bonded negatively charged polyacrylamide gel-filled capillary was evaluated for use as an optical purity test, which is important in the pharmaceutical industry. The desirable lower limit of

Table 2
Within- and between-run reproducibilities of retention time and separation factor

Compound	RSD (%, $n=6$[c])					
	Within-run			Between-run		
	t_1	t_2	α	t_1	t_2	α
1-(1-Naphthyl)ethylamine[a]	2.9	3.2	0.3	1.5	1.3	0.2
Tryptophanol[b]	0.7	0.8	0.2	1.8	2.1	0.5
Alanine-2-naphthylamide[b]	0.7	1.3	0.9			

[a] Conditions: chiral selector concentration, 20 m*M*; mobile phase, 200 m*M* triethanolamine–300 m*M* boric acid buffer (pH 6.0); applied voltage (current), +125 V cm^{-1} (5 μA). Other conditions as in Table 1.
[b] Conditions as in Table 1.
[c] n is the total number of measurements carried out.

Table 3
Performance of three different columns[a]

Column No.	Tryptophanol (average, $n=6$[b])			Alanine-2-naphthylamide (average, $n=6$[b])		
	t_1 (min)	t_2 (min)	α	t_1 (min)	t_2 (min)	α
1	29.7	31.2	1.05	26.9	33.0	1.23
2	30.4	31.5	1.04	27.4	33.1	1.21
3	33.8	34.9	1.03	25.7	29.9	1.16

[a] Conditions as in Table 1.
[b] n is the total number of measurements carried out.

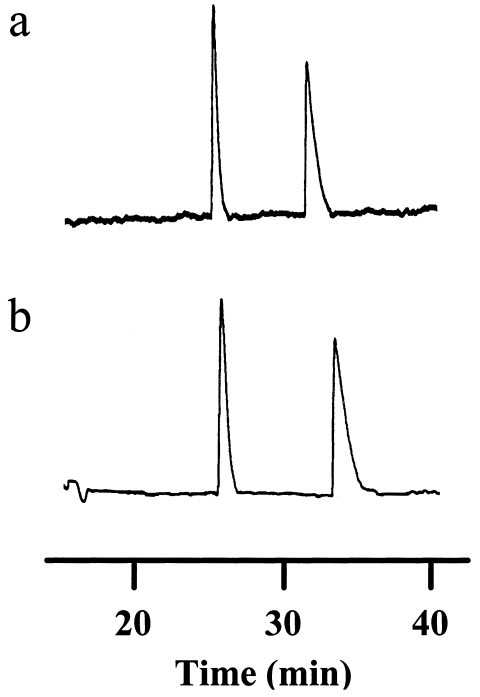

Fig. 5. Stability of a (+)-tetraallyl 18-crown-6 carboxylate-bonded negatively charged polyacrylamide gel-filled capillary. Electrochromatograms of alanine-2-naphthylamide obtained immediately after preparation of the column (a), and after intermittent use for 3 months followed by storage at room temperature for 10 months (b). Conditions as in Table 1.

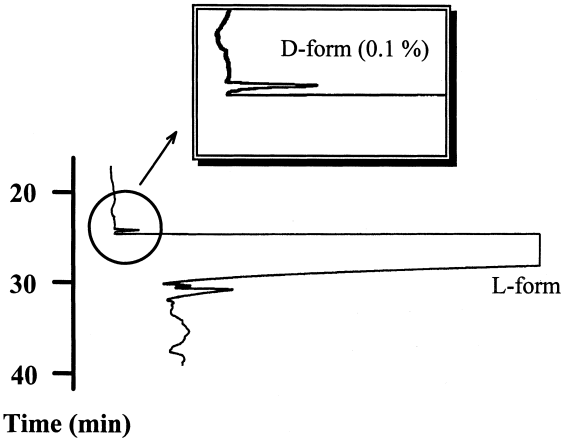

Fig. 6. Optical purity test of L-alanine-2-naphthylamide with a (+)-tetraallyl 18-crown-6 carboxylate-bonded negatively charged polyacrylamide gel-filled capillary. Sample: L-alanine-2-naphthylamide spiked with ca. 0.2% of DL-alanine-2-naphthylamide. Conditions: applied voltage (current), 239 V cm^{-1} (6 μA). Other conditions as in Table 1.

quantitation is usually 0.1% in the pharmaceutical industry. Using the same mobile phase as in Table 1, the optical purity determination of L-alanine-2-naphthylamide was performed. As can be seen from the electrochromatogram of L-alanine-2-naphthylamide shown in Fig. 6, 0.1% of the minor enantiomer (D-form) is detectable by our CEC method. Consequently, it may be possible for our method to determine 0.1% of the minor enantiomer. In this case this low detection level would be ascribed to the high R_s value in the enantiomeric separation of alanine-2-naphthylamide.

3.3. (+)-18-Crown-6 tetracarboxylic acid 2-allyl ester-bonded negatively charged polyacrylamide gel-filled capillary

3.3.1. Enantiomeric separation of primary amino compounds

Enantiomeric separations of various primary amino compounds were investigated using (+)-18-crown-6 tetracarboxylic acid 2-allyl ester-bonded negatively charged polyacrylamide gel-filled capillaries. As a result of the analyses, enantiomeric separations of nine primary amino compounds were achieved using 200 m*M* triethanolamine–300 m*M* boric acid buffer (pH 7.0) as a mobile phase. The results are summarized in Table 4. High efficiencies of more than 100 000 plates m^{-1} were obtained for alanine-2-naphthylamide, tryptophanol and 2-amino-9-hydroxyfluorene. Typical electrochromatograms of tryptophanol and 1,2,3,4-tetrahydro-1-naphthylamine are shown in Fig. 7. The EOF velocity was 8.8 cm min^{-1} when DMSO as an unretained solute marker was analyzed at an applied voltage of +200 V cm^{-1}.

3.3.2. Reproducibility and stability

The within- and between-run reproducibilities of retention time and separation factor were examined for 1-(1-naphthyl)ethylamine, 1,2,3,4-tetrahydro-1-naphthylamine, tryptophanol and 2-amino-9-hydroxyfluorene. The RSDs of the within- and between-run reproducibilities of retention time were less than 2.6 and 4.9% over the six injections, respectively. Those of the separation factor were less than 1.4 and 2.2%, respectively. The reproducible results obtained are summarized in Table 5.

Table 4
Enantiomeric separation of primary amino compounds with (+)-18-crown-6 tetracarboxylic acid 2-allyl ester-bonded negatively charged polyacrylamide gels[a]

Compound	Retention time (min)		Plate number (m^{-1})		α	R_s
	t_1	t_2	N_1	N_2		
1-(1-Naphthyl)ethylamine	25.6	27.0	41 000	27 000	1.05	1.27
Alanine-2-naphthylamide	34.2	36.7	121 000	96 000	1.07	3.12
Methionine-2-naphthylamide	48.7	55.8	62 000	44 000	1.15	3.95
1-Aminoindan	17.8	21.0	39 000	8000	1.18	2.41
1,2,3,4-Tetrahydro-1-naphthylamine	18.8	20.3	37 000	17 000	1.08	1.29
1,2-Diphenylethylamine	27.1	29.3	7000	2000	1.08	0.61
2-Amino-1,2-diphenylethanol	24.1	28.5	14 000	4000	1.18	1.55
Tryptophanol	22.5	23.2	125 000	108 000	1.03	1.40
2-Amino-9-hydroxyfluorene	39.3	40.7	117 000	78 000	1.03	1.42

[a] Conditions: capillary, fused-silica capillary [75 m (effective length 35 cm)×75 μm I.D.]; %T, 5.0; %C, 5.0; %S 5.5; mobile phase, 200 m*M* triethanolamine–300 m*M* boric acid buffer (pH 7.0); chiral selector, 20 m*M*; applied voltage (current), +200 V cm^{-1} (9 μA); detection wavelength, 254 nm; sample concentration, 1–10 mM; sample injection, electrokinetic method.

3.4. Comparison between the (+)-tetraallyl 18-crown-6 carboxylate and (+)-18-crown-6 tetracarboxylic acid 2-allyl ester-bonded negatively charged polyacrylamide gel-filled capillaries

As shown in Tables 1 and 4, the seven primary amino compounds [1-(1-naphthyl)ethylamine, alanine-2-naphthylamide, methionine-2-naphthylamide, 1-aminoindan, tryptophanol, 1,2-diphenylethylamine, 2-amino-1,2-diphenylethanol] were separated enantiomerically with each of the two kinds of gel-filled capillaries. On the other hand, the enantiomeric separations of 1-phenylethylamine, α-methyltryptamine and tryptophan methyl ester were obtained only with the (+)-tetraallyl 18-crown-6 carboxylate-bonded negatively charged polyacrylamide gel-filled capillary, and those of 1,2,3,4-tetrahydo-1-naphthylamine and 2-amino-9-hydroxyfluorene were obtained only with the (+)-18-crown-6 tetracarboxylic acid 2-allyl ester-bonded negatively charged polyacrylamide gel-filled capillary. These results indicate that it is useful to have the two kinds of gel-filled capillaries because more primary amino compounds can be separated enantiomerically when both are used.

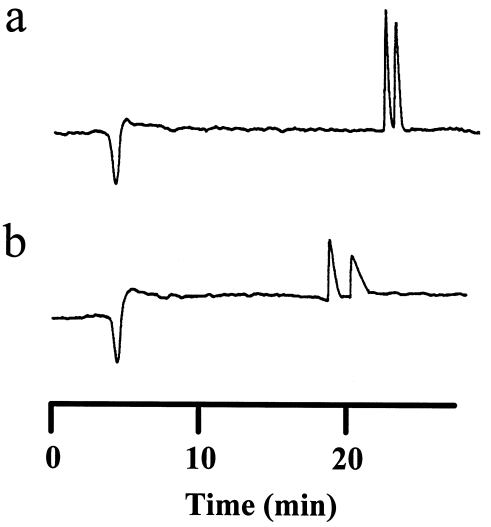

Fig. 7. Enantiomeric separation of tryptophanol (a) and 1,2,3,4-tetrahydro-1-naphthylamine (b) with (+)-18-crown-6 tetracarboxylic acid 2-allyl ester-bonded negatively charged polyacrylamide gel-filled capillary. Conditions as in Table 4.

4. Conclusion

The enantiomeric separations of various primary amino compounds were achieved using capillaries filled with chiral crown ether-bonded negatively charged polyacrylamide gels. High efficiencies of more than 100 000 plates m^{-1} were obtained for some 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

Table 5
Within- and between-run reproducibilities of retention time and separation factor[a]

Compound	RSD (%, $n=6$[b])					
	Within-run			Between-run		
	t_1	t_2	α	t_1	t_2	α
1-(1-Naphthyl)ethylamine	1.8	1.7	0.2	4.3	4.9	0.9
1,2,3,4-Tetrahydro-1-naphthylamine	1.3	2.6	1.4	3.4	4.0	1.1
Tryptophanol	1.8	1.8	0.2	2.2	2.3	0.4
2-Amino-9-hydroxyfluorene	2.1	2.1	0.1	2.7	2.8	2.2

[a] Conditions as in Table 5.
[b] n is the total number of measurements carried out.

used during the polymerization process. The gel-filled capillaries prepared were stable to intermittent use for 3 months followed by storage at room temperature for 10 months. From the results of the optical purity test of L-alanine-2-naphthylamide, 0.1% of the minor enantiomer is detectable by our CEC method. We have described a simple and reliable method for enantiomeric separation by CEC in this paper. The present CEC method should be useful with regard to immobilizing the expensive chiral crown ethers and no frits to keep the stationary phase in place. It may be possible to obtain better results of enantiomeric separations of more primary amino compounds, shorter analysis time and better resolution with further optimized the gel composition and separation conditions in the future.

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