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Enantioseparation using selected polysaccharides as chiral buffer additives in capillary electrophoresis

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Abstract

Selected water-soluble, native polysaccharides – such as amylose, laminaran, pullulan – and derivatized polysaccharides – methyl cellulose, hydroxypropyl cellulose, and carboxymethyl amylose sodium salt (CM-Am) – were investigated as chiral selectors in capillary electrophoresis. Effects of degree of polymerization and concentration of amylose on the separation of enantiomers of 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (BDHP) and a chiral cardiovascular drug *cis*-diltiazem were also studied. Pullulan and amyloses used in this study showed the same migration order for enantiomers of BDHP. In contrast, the migration order of BDHP enantiomers for cellulose derivatives and laminaran as well as with β-cyclodextrin was opposite to that for amylose and pullulan. The enantioseparation of several chiral drugs was also performed using high-molecular-mass amylose (Am-4900) and CM-Am.

Keywords: Buffer composition; Enantiomer separation; Polysaccharides; Diltiazem; Binaphthyldiyl hydrogen phosphate

1. Introduction

Chiral compounds containing sugar units play a key-role as chiral selectors in capillary electrophoresis (CE). The extensively studied chiral selectors are cyclic oligosaccharides, cyclodextrins (CDs) and their derivatives [1–4], linear oligosaccharides [1,3,5–9], glycopeptides [10–15], natural glycoside-type surfactants [16,17], sulfated natural polysaccharides such as heparin [18–21], dextran sulfate [20–22] and chondroitin sulfate [20,21], neutral polysaccharides dextran and dextrin [23–25] and glucopyranoside-based surfactants [26]. However, the chiral recognition ability of the most abundant natural polysaccharides such as cellulose and amylose still remains unexplored in chiral CE separations. Moreover, the chiral recognition ability of some water soluble cellulose derivatives is to some extent neglected so that they are used as 'achiral' buffer additives in chiral CE to modify electroosmotic flow (EOF) and to diminish interaction of analytes with the capillary inner wall [27–31].

Although some natural sulfated [18–22] and neutral [25] polysaccharides were used as chiral selectors in CE, the effects of degree of polymerization (DP) and type of linkage between saccharide units on chiral recognition are not clear at present.

D'Hulst and Verbeke [5] observed no measurable chiral resolving ability of oligo- and polysaccharides with monomer linkages other than α-(1-4) in CE.

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They concluded that chains of D-glucose units linked through α-(1-4) bonds play a key-role in chiral recognition. Later, Novotny and co-workers [6,8] used Dextrin 10 [Glu-(1-4)-α-D-Glu], dextran [Glu-(1-6)-α-D-Glu], laminarin [dominantly Glu-(1-3)-β-D-Glu] and alginic acid together with glucose and non-cyclic maltooligosaccharides (DP=2–7) as chiral selectors and confirmed the importance of the α-(1-4) linkage for the chiral recognition. In contrast to their results, Nishi and co-workers showed that dextran sulfate [22] and dextran [24,25] with mainly α-(1-6) glycoside bonds were also useful chiral selectors for the separation of some chiral compounds.

The effect of DP of oligo- and polysaccharides on chiral recognition still remains scarcely studied [5,8,25]. Nishi et al. [25] observed a weak effect of DP on the chiral recognition ability of dextran and dextrin. D'Hulst and Verbeke [5] found that maltooligosaccharide mixtures of a lower DP range were ineffective in enantioseparation of some 2-arylpropionic acid derivatives, while high DP corn syrup subfractions were effective for chiral separation. A more detailed study on the effect of DP on chiral recognition ability of maltooligosaccharides was done by Soini et al. [8]; they observed an increasing enantioselectivity with an increase in molecular mass of the maltooligosaccharides, and postulated that a helical structure mimicking a cyclodextrin cavity would be responsible for the chiral recognition. Kano et al. [9] also suggested the importance of a helical structure of non-cyclic oligosaccharides for chiral recognition in CE.

The objective of the present study is to investigate the chiral recognition abilities of water-soluble, native amylose with various DPs, carboxymethyl amylose (CM-Am), water-soluble, low viscosity methyl cellulose (MEC) and hydroxypropyl cellulose (HPC), and of natural polysaccharides, i.e. laminaran and pullulan, with emphasis on the effects of linkage type between the saccharide units and DP on the chiral recognition ability in CE. In addition the usefulness of high-molecular-mass, water-soluble native amylose (Am-4900) for chiral CE separation of several drugs was demonstrated for the first time.

2. Experimental

2.1. Instrumentation

CE experiments were performed using a Prince Model CE instrument (Lauerlabs, Emmen, Netherlands) equipped with a variable-wavelength Jasco CE-871 UV detector (Jasco, Tokyo, Japan). Detection was performed at 210 nm. Fused-silica capillaries (50 μm I.D., 360 μm O.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA). Separation conditions are shown in the figure captions.

Enantioseparations were evaluated with separation factor (α), peak efficiency (N) and resolution (R_s). The separation factor α and R_s were calculated from t_2/t_1 and $2(t_2-t_1)/(w_1+w_2)$, respectively, where t_1 and t_2 are the migration times of the first and second enantiomers and w_1 and w_2 are the corresponding widths at the peak base. The concentration of the samples was ca. 0.1 mg/ml. A 2:1 mixture of (*S*)- and (*R*)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (BDHP) was used to determine the migration order of the enantiomers.

2.2. Chemicals and reagents

Maltoheptaose (M7) was obtained from YMC (Kyoto, Japan). Water-soluble maltooligosaccharide with DP=18 (M18) and amyloses with DP=4900 (Am-4900), DP=6250 (Am-6250) and DP=16250 (Am-16250) were kindly supplied from Nakano Vinegar (Handa, Japan). The solubility of Am-4900 is 7–10 mg/ml in triethtanolamine-phosphate or sodium phosphate buffer in the pH range 3.0–7.0. Methyl cellulose (MEC, viscosity 13–18 cps) and hydroxypropyl cellulose (HPC, viscosity 3–7 cps) were purchased from Nakalai Tesque (Kyoto, Japan). Carboxymethyl amylose (sodium salt) (CM-Am), pullulan, and racemic mianserine, *cis*-diltiazem, ibuprofen, and trihexyphenidyl were from Sigma (St. Louis, MO, USA). Tröger's base, BDHP and its optically pure enantiomers were obtained from Aldrich (Milwaukee, WI, USA). Laminaran (from Eisenia bicycles), mono- and dibasic sodium hydrogen phosphates, phosphoric acid, triethanolamine,

sodium hydroxide and hydrogen chloride were from Tokyo Kasei (Tokyo, Japan).

3. Results and discussion

3.1. Chiral recognition of BDHP by polysaccharides

Native amylose and cellulose are usually insoluble in water, which may be the main reason for the limited use of these polymers as chiral selectors in CE where aqueous buffers are commonly used. However, it is well known that high-molecular-mass amylose is soluble in water [32]. This material can be used as a chiral selector in CE. Water-soluble amyloses were prepared by enzymatic polymerization of α-D-glucose 1-phosphate dipotassium catalyzed by a phosphorylase [32]. Some water-soluble cellulose derivatives are commercially available.

The separation results of a nonracemic mixture of (*R*)-(−)- and (*S*)-(+)-BDHP using Am-4900, MEC, HPC, laminaran and pullulan (Fig. 1) as chiral selectors are shown in Fig. 2. All the polysaccharides exhibited chiral recognition ability toward BDHP in an anionic form at pH 3. Especially, Am-4900 showed high chiral recognition ability (Fig. 2a) comparable to that of β-CD which is among the most useful chiral selectors in CE. As shown in Fig. 2b and Fig. 2c, the alkyl- and hydroxyalkyl-cellulose which are often used as 'achiral' buffer additives in chiral CE [27–31], also exhibited chiral recognition ability. Laminaran which mainly consists of β-(1-3)-D-polyglucan also showed chiral recognition toward BDHP enantiomers (Fig. 2e), although it was reported that laminaran did not show chiral recognition ability to other chiral compounds [6].

Am-4900 consisting of D-glucose units linked by α-(1-4) bonds and pullulan consisting of α-(1-4) linked and α-(1-6) linked D-glucose (2:1) bound preferentially (*R*)-(−)-BDHP, while polysaccharides linked by β-(1-4) or β-(1-3) bonds interacted preferentially with the (*S*)-(+)-BDHP. These results are in good agreement with those in the enantioseparation using disaccharides as a chiral selector [33]; both α-(1-4) and α-(1-6) linked disaccharides bind preferentially (*R*)-(−)-BDHP, whereas β-(1-4) linked disaccharides (*S*)-(+)-BDHP. The type of linkage may play a role for the chiral recognition.

3.2. pH dependence of enantiomer migration order of BDHP

At pH 3, the phosphoric acid residue of BDHP is deprotonated. Therefore, in the bare silica capillary, the EOF is low at this pH and the chiral solute was injected at the cathodic end of the capillary and detected at the anodic side. Under these conditions,

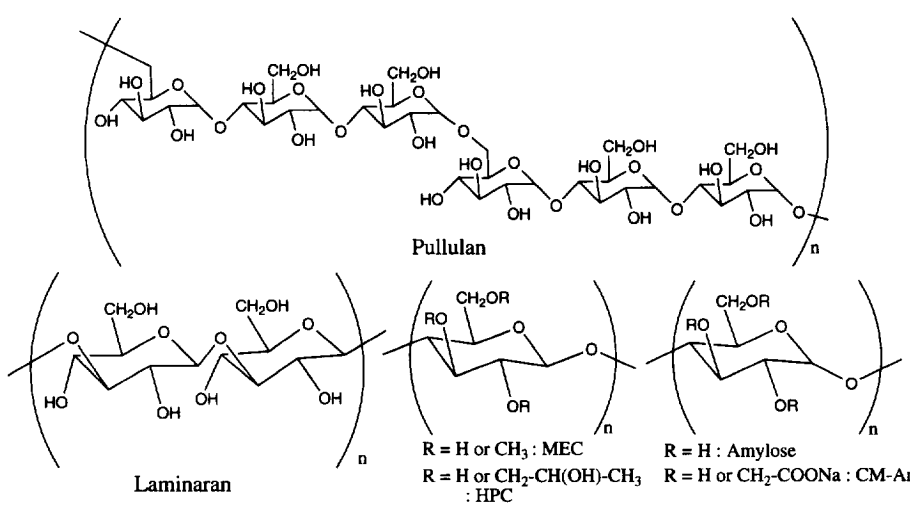

Fig. 1. Structures of polysaccharides.

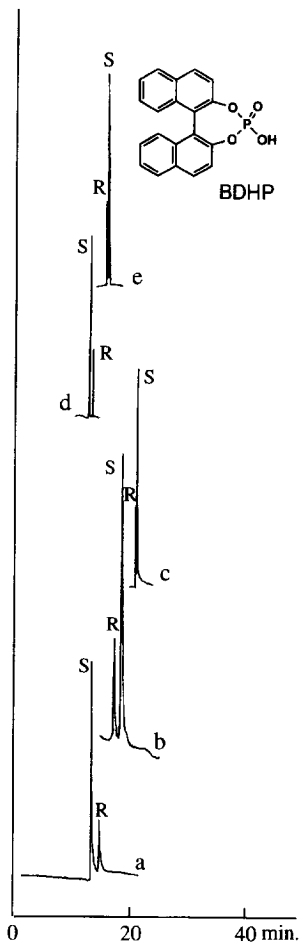

Fig. 2. Enantioseparation of nonracemic mixtures of (*S*)-(+)- and (*R*)-(−)-BDHP using Am-4900 (5 mg/ml) (a), methyl cellulose (20 mg/ml) (b), hydroxypropyl cellulose (20 mg/ml) (c), pullulan (20 mg/ml) (d) and laminaran (20 mg/ml) (e). Conditions: bare silica capillary (50 μm I.D.) with 60 cm total length and 47 cm effective length; 50 m*M* phosphate buffer (pH 3.0); applied voltage, 20 kV; sample injection with pressure at the cathodic end of the capillary.

the enantiomer of BDHP preferentially complexed with the chiral selector will be expected to migrate as the second peak.

By increasing the pH of background electrolyte the EOF in a bare silica capillary increases and when it exceeds the electrophoretic mobility of BDHP, reversal of the polarity of the high voltage supply is required to achieve detection of the analyte. This results in a reversal of the enantiomer migration order, though the chiral recognition pattern of the

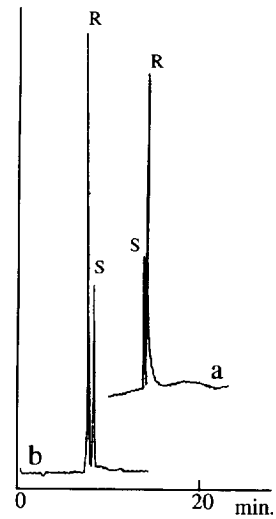

Fig. 3. Enantioseparation of nonracemic mixtures of (*S*)-(+)- and (*R*)-(−)-BDHP using Am-4900 (5 mg/ml). 50 m*M* phosphate buffer at pH 3.0 (a) and pH 7.0 (b). Sample injection with pressure at the cathodic (a) and anodic (b) ends of the capillary. Other conditions as shown in Fig. 2.

chiral selector remains the same [34]. This is demonstrated in Fig. 3 using Am-4900 as a chiral selector. The advantage of reversal of the enantiomer migration order for determination of trace amounts of enantiomeric impurities is well documented in chiral CE [35,36].

3.3. Comparison of chiral recognition ability of polysaccharides and cyclodextrins

The general hypothesis regarding the chiral recognition mechanism of non-cyclic oligosaccharides and polysaccharides is that a pseudocyclic structure mimicking CDs may be responsible for their efficient chiral recognition ability [8,9].

The results on the separation of BDHP with Am-4900, MEC, HPC and β-CD [34] may not support the hypothesis. Am-4900 containing α-(1-4) linked D-glucose units was expected to mimic the β-CD cavity. However, Am-4900 and β-CD exhibited an opposite chiral recognition ability to the enantiomers of BDHP. Am-4900 binds preferentially the (*R*)-(−)-BDHP, while β-CD binds preferentially the (*S*)-(+)-BDHP [34]. On the other hand, MEC and HPC, consisting of β-(1-4)-linked D-glucose units, cannot mimic the β-CD cavity. However, both cellulose

derivatives displayed the same recognition pattern towards the BDHP enantiomers as β-CD.

Moreover, Am-4900 resolved the enantiomers of *cis*-diltiazem, whereas α-, β- and γ-CDs could not resolve the enantiomers even at a concentration four-fold that of Am-4900 (Fig. 4).

3.4. Effect of DP on the chiral recognition ability of amylose towards BDHP

The DP may affect the chiral recognition ability of polymeric type chiral selectors. Generally, it is considered that higher-molecular-mass polymers may show higher chiral recognition ability due to the formation of higher order secondary structure of chiral polymers [5,8]. However, recently, Nishi et al. [25] reported no significant change in separation factor (α) and peak resolution with increasing molecular mass of dextrin, and no change of selectivity and slight increase of peak resolution were observed with increasing molecular mass of dextran, in the range 40 000–300 000.

The amylose used in this study had narrow molecular mass distribution ($M_w/M_n<1.1$) [32]. Additionally, these amyloses are water-soluble. This made it possible to study the effect of DP on chiral recognition ability (Table 1).

Table 1
Influence of degree of polymerization (DP) of amylose on the separation factor (α) for enantiomers of BDHP and *cis*-diltiazem[a]

DP	α	
	BDHP	*cis*-diltiazem
7	1.00	1.00
18	1.02	1.00
4900	1.03	1.01
6250	1.03	1.01
16 250	1.00	1.00

[a] Separation conditions: 50 μm (I.D.) fused-silica capillary with 48 cm effective length and 60 cm total length; applied voltage, 20 kV; sodium dihydrogen phosphate buffer at pH 3.0 containing 2.2 mg/ml chiral selector. Samples were injected at cathodic end of the capillary in case of BDHP and at anodic end in case of *cis*-diltiazem.

As shown in Table 1, the enantioselectivity for BDHP and *cis*-diltiazem was strongly dependent on the DP of amylose. Maltoheptaose as well as the highest-molecular mass amylose (Am-16250) showed no enantioselectivity at the concentration (2.2 mg/ml) of the chiral selectors. The reason for the unusual molecular-mass dependenece in the chiral recognition of amylose is not clear at present.

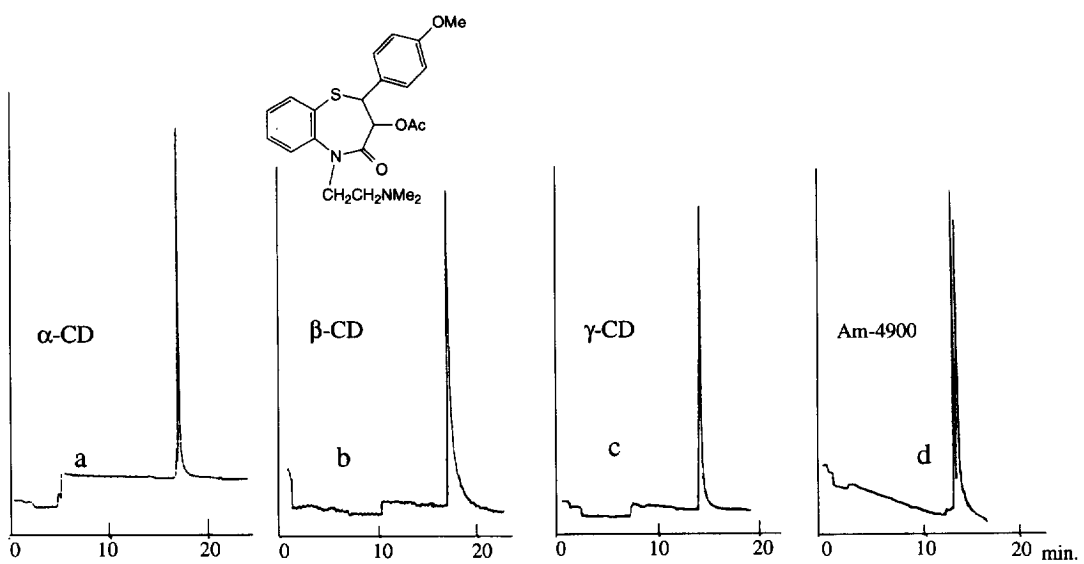

Fig. 4. Enantioseparation of racemic *cis*-diltiazem hydrochloride with α- (a), β- (b), γ- CDs (c) and Am-4900 (d). All CDs were used as buffer additives in concentration of 16 mg/ml, and Am-4900 in concentration of 4 mg/ml. Samples were injected with the pressure at the anodic end of the capillary. Other conditions as shown in Fig. 2.

Recently, Agyei et al. [37] also reported that the intermediate-molecular-mass dextran sulfate gave the highest resolution for chloroquine enantiomers. Although the poor chiral recognition of maltoheptaose may be attributed to the absence of higher order structure (i.e., a helix) and effective hydrophobic interaction sites, more detailed binding studies should be performed in order to explain the observed effect. However, maltoheptaose as well as even lower DP malto- and cellooligosaccharides exhibit chiral resolving ability toward BDHP at relatively high concentrations [33]. Kano et al. [9] also reported the enantioseparation of BDHP using maltooligosaccharides in CE.

3.5. Effect of concentration of Am-6250 on the enantioseparation of BDHP and cis-diltiazem

The amylose with DP=6250 (Am-6250) is more soluble in aqueous buffers than the other amyloses used in this study. This amylose was used to investigate the effect of concentration on the enantioseparation of BDHP and *cis*-diltiazem.

As shown in Fig. 5, α and the R_s showed a general trend for both the chiral compounds; α and R_s increased with an increase in the concentration of Am-6250 in the background electrolytes and reached a maximum at a chiral selector concentration of about 15 mg/ml, and then decreased with a further increase of the polysaccharide concentration. The sharp decreases of the separation factor as well as the peak resolution at concentrations above 15 mg/ml indicate that the study in the higher concentration range must be done. This was impossible due to the extremely high viscosity of the solutions. A similar change of resolution factor was also reported for the separation of chlorpheniramine and chloroquine enantiomers with dextran sulfate [37].

Optimum concentrations for both α and R_s are often observed in chiral CE separations and this is supported by the model proposed by Wren and Rowe [38]. However, further studies are required in order to examine the underlying mechanism in more detail.

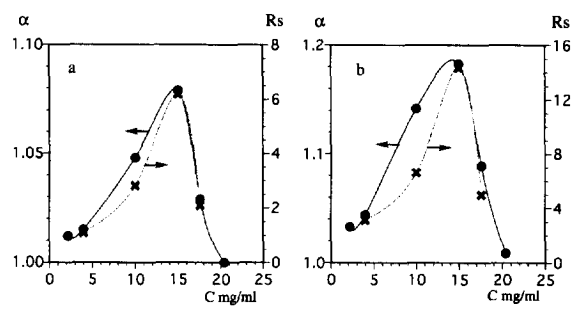

Fig. 5. Dependence of the separation factor (α) and resolution (R_s) for *cis*-diltiazem (a) and BDHP (b) enantiomers on the concentration of Am-6250 in the background electrolyte. Other conditions as in Table 1.

3.6. Use of water soluble amyloses (Am-4900 and CM-amylose) as chiral selectors in CE

In order to examine the usefulness of the water-soluble amyloses as chiral selectors in CE, they were used for the enantioseparation of several basic or acidic compounds. Examples of the separation of the racemic cholinergic drug trihexyphenidyl, Tröger's base, antihistaminic drug, mianserine, and nonsteroidal antiinflammatory drug, ibuprofen, are shown in Fig. 6.

Am-4900 seems to be a candidate for an effective chiral selector in CE, but the solubility of this polysaccharide in aqueous solutions is low (5–10 mg/ml at pH 3–7), which may limit its application for practical purposes. As already demonstrated for CDs, the derivatization of hydroxy groups by alkyl, hydroxyalkyl or ionic substituents (carboxyalkyl, sulfo- or sulfoalkyl, etc.) can substantially increase the solubility without a decrease, and in many cases even with an increase of the chiral recognition ability [1–4]. Derivatization with ionic groups proved to be more effective in the case of CDs [4]. The solubility of ionic derivatives in aqueous buffers is much higher than that of non-ionic derivatives. Ionic derivatives also possess additional groups for electrostatic interactions with oppositely charged analytes and for hydrogen bonding. Another important advantage of ionic derivatives is that, in contrast to neutral polysaccharides, they possess electrophoretic mobility which allows their use in the capillary electrokinetic chromatographic mode for the resolution of neutral compounds. A preliminary study of a commercially available CM-Am showed that the ionic amylose derivatives may have potential as chiral selectors in CE (Fig. 7).

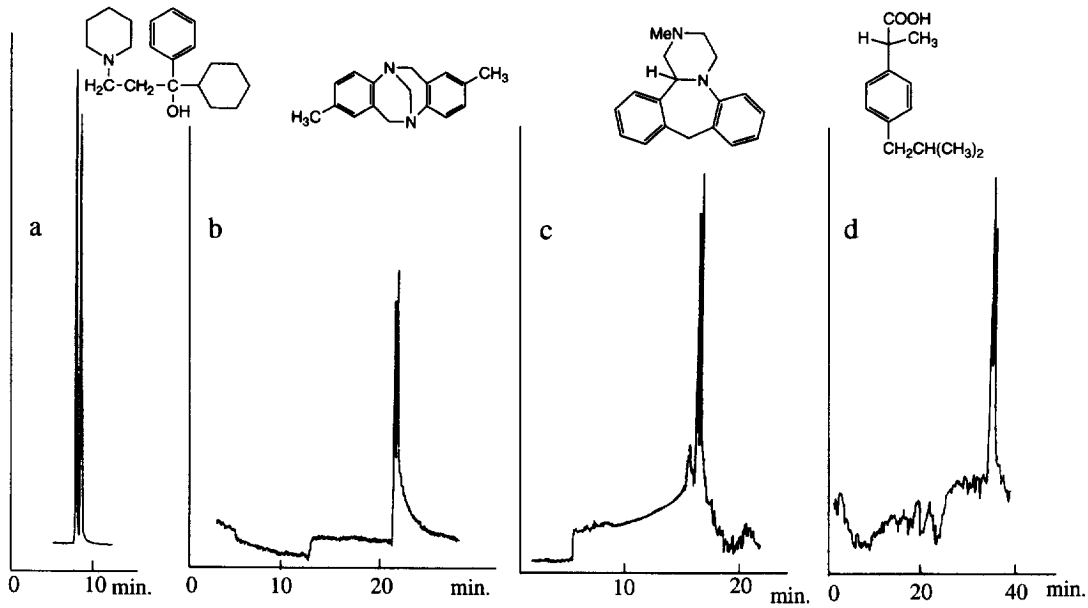

Fig. 6. Enantioseparation of racemic trihexyphenidyl (a), Tröger's base (b), mianserine (c) and ibuprofen (d) using 10 mg/ml Am-4900 as buffer additive. 100 m*M* H_3PO_4-triethanolamine buffer at pH 3.0 for a, b and c and at pH 7 for d. Samples were injected at the anodic end of the capillary for a, b and c and at the cathodic end of the capillary for d. Other conditions as in Fig. 2.

Further study on enantioseparation of other chiral compounds is in progress.

Acknowledgments

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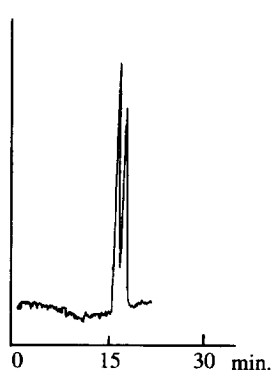

Fig. 7. Enantioseparation of racemic *cis*-diltiazem hydrochloride with 10 mg/ml CM-amylose as a buffer additive. Other conditions as in Fig. 4.

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