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Review

Enantioselectivity in chiral capillary electrophoresis with polysaccharides

Hiroyuki Nishi

Analytical Research Laboratory, Tanabe Seiyaku Co., Ltd., 16-89, Kashima 3-chome, Yodogawa-ku, Osaka 532, Japan

Abstract

This review surveys enantiomer separation by capillary electrophoresis (CE) using polysaccharides as chiral selectors. Many ionic or electrically neutral polysaccharides, such as heparin, chondroitin sulfate, dextrin, etc., have been employed successfully for the CE separation of enantiomers. The operational conditions that affect the enantioselectivity of the chiral separation system will be described. The mechanism of enantioseparation will also be discussed briefly. © 1997 Elsevier Science B.V.

Keywords: Reviews; Enantiomer separation; Polysaccharides

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1. Introduction

Enantiomer separation by capillary electrophoresis (CE) has rapidly attracted attention as a promising technique, especially in the field of pharmaceutical sciences. Typically enantiomer separations by CE are achieved with one capillary tube (without a special column and an organic solvent), with high resolution and relatively fast separation. Furthermore, the amounts of sample and media required in CE techniques are extremely small. Among the various CE modes, capillary zone electrophoresis (CZE) and electrokinetic chromatography (EKC), where only a chiral selector is added to the usual running buffer solution, are the most widely used for enantiomer separation [1–5].

One of the most attractive advantages of CZE and EKC for the separation of enantiomers is that the separation media can be changed easily in method development, that is, one can easily alter the separation solution to find the optimum separation conditions and an expensive chiral selector could also be used because of the small media requirement. Electrically neutral chiral selectors are employed for the enantiomer separation of ionic solutes (CZE mode). Charged pseudo-stationary phases, such as micelles and proteins, are employed for the separation of enantiomers by EKC. Electrically neutral enantiomers as well as ionic ones can be separated in the EKC mode [5].

Cyclodextrins (CDs), which are cyclic oligosaccharides and were first employed by Fanali in 1989 [6,7], have been found to be most effective in the CZE enantiomer separation of a wide range of ionic drugs [3,4,8]. Besides CDs, many other compounds containing sugar units as the chiral moiety have been successfully employed for enantiomer separation in CE [9–15]. Glycopeptides, such as vancomycin and ristocetin A [9–11], some saponins, such as β-escin [12] and glycyrrhizic acid [12], which are also natural surfactants, and glucopyranoside-based synthesized surfactants [13–15] have been found to be useful as chiral selectors in CE. Sugar units play a key role in enantiorecognition, e.g. in high-performance liquid chromatography (HPLC) [16,17]. Especially in enantiomer separation by CE, these oligo- and polysaccharides are advantageous because they have good UV transparency. Charged polysaccharides are have good solubility in aqueous solutions.

The first CE enantiomer separation using carbohydrates alone as chiral selectors was reported by D'Hulst and Verbeke in 1992 [18]. They employed various maltodextrins that had a low degree of polymerization (DP, defined as the number of saccharide monomers within the oligo- or polysaccharide chain). From studies on a series of maltodextrins, it was found that the higher the DP number, the higher was the enantioresolving capability [18]. They extensively investigated CE enantiomer separation using oligosaccharides such as Glucidex 2 (dextrose equivalent number, DE, defined as the percentage of reducing sugars calculated as glucose on a dry-substance basis; DE=2) [19,20] and Glucidex 6 (DE=6) [21]. Dextrin 10 (DE=10) was also successfully employed for enantiomer separation [22,23]. Recently, dextrin 10 sulfopropyl ether was prepared and employed for CE enantiomer separation [24]. After the work of D'Hulst and Verbeke, many ionic or neutral polysaccharides have been employed for CE enantiomer separation [25,26].

In this paper, CE enantiomer separations using polysaccharides, including CD polymers, as chiral selectors, are reviewed. The operational conditions that affect the enantioselectivity of the enantiomer separation system will be mentioned. The separation mechanism will also be discussed briefly.

2. Enantiomer separation by capillary electrophoresis with polysaccharides

In the CE separation of enantiomers using polysaccharides, polysaccharides are added to the running buffer solution. The conditions for optimal enantiomer separation can be manipulated by changing the buffer solution containing the polysaccharide chiral selector. In this case, the pH of the buffer and the concentration of the chiral selector (polysaccharide) are two important factors affecting the enantioselectivity, other than the species of the chiral selector.

When electrically neutral polysaccharides are used, only ionic enantiomers can be separated (CZE mode). On the other hand, electrically neutral enantiomers as well as ionic ones can be separated

Table 1
Ionic or chargeable polysaccharides employed for CE enantiomer separation

Name	Molecular mass	S content	SO_4^{2-} (per disaccharide)	Main monosaccharide unit	Main linkage
Heparin	7000–20 000 DP>200	ca. 11%	2.0–3.0	D-Glucuronic acid *N*-acetyl-D-glucosamine	α(1→4)
Chondroitin sulfate C	30 000–50 000	ca. 7%	0.2–2.3	D-Glucuronic acid *N*-acetyl-D-galactosamine (6-sulfate)	β(1→3)
Chondroitin sulfate A	30 000–50 000 DP: 80–130	ca. 7%	0.2–2.3	D-Glucuronic acid *N*-acetyl-D-galactosamine (4-sulfate)	β(1→3)
Dextran sulfate	7300	ca. 18%	6	D-Glucose (isomaltose)	α(1→6)
λ-Carrageenan	–	ca. 12%	2.0–2.7	D-Galactose	β(1→4) α(1→3)
CM–amylose	–	–	–	D-Glucose (maltose)	α(1→4)
DEAE–dextran	500 000	–	–	D-Glucose (isomaltose)	α(1→6)

when charged polysaccharides (EKC mode) are used. The electrically neutral and charged polysaccharides that are commonly used are summarized in Tables 1 and 2, as are some of their physico-chemical properties [27]. The unit structures of these polysaccharides are shown in Figs. 1 and 2 [27]. Most of them are naturally occurring polysaccharides and have a wide molecular mass range. Therefore, natural polysaccharides from different sources can give changes in enantioselectivity due to the molecular mass distribution and the different ratios of the unit components.

In most cases, a capillary washing process is required for CE enantiomer separation using polysac-

Table 2
Neutral polysaccharides employed for CE enantiomer separation

Name	Molecular mass	Monosaccharide unit	Linkage
Dextrin	Achrodextrin ca. 3700 Erythrodextrin 6200–7000 Amylodextrin >10 000	D-Glucose (maltose)	α(1→4)
Amylose	50 000–150 000 DP: 240–3800	D-Glucose (maltose)	α(1→4)
Dextran	~ ca. 4 000 000 (native dextran) DP: 370–300 000	D-Glucose (isomaltose)	α(1→6)
Cellulose (MEC, HPC)	230 000–600 000 DP: 3000–570 000	D-Glucose (cellobiose)	β(1→4)
Laminaran	DP: 20–30	D-Glucose (laminaribiose)	β(1→3)
Pullulan	DP: 1500	D-Glucose (maltose) (isomaltose)	α(1→4) α(1→6)

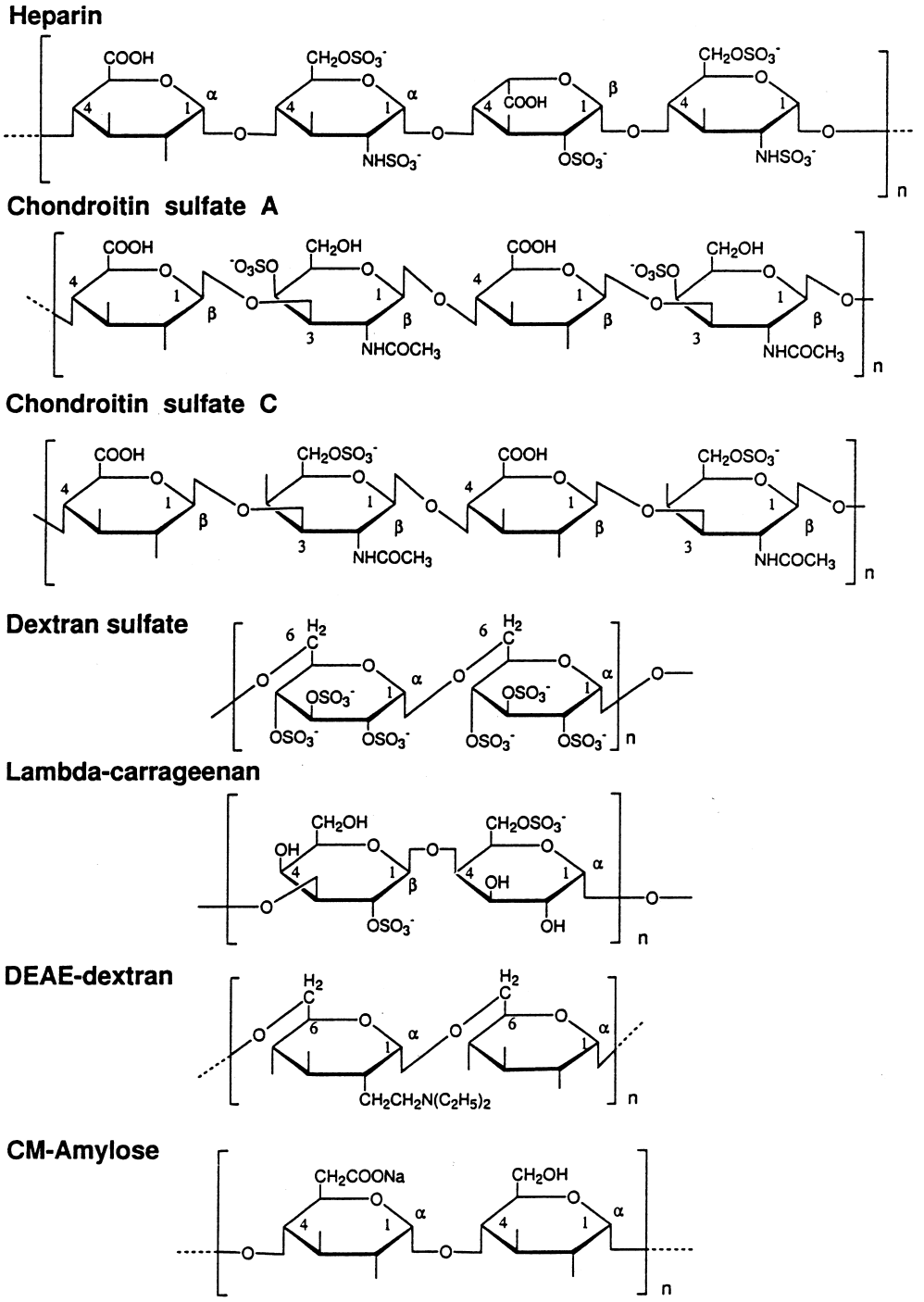

Fig. 1. Unit structures of ionic polysaccharides employed for CE enantiomer separations.

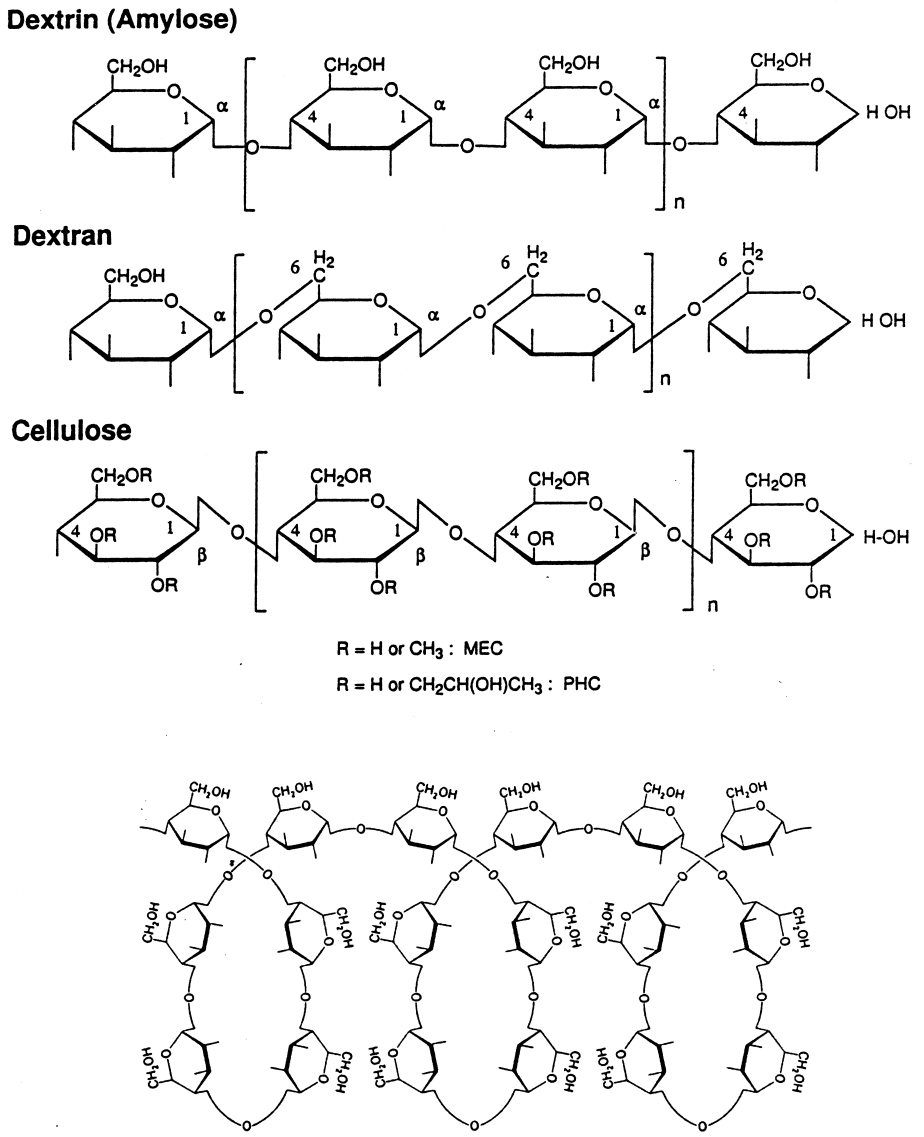

Fig. 2. Unit structures of neutral polysaccharides employed for CE enantiomer separations. A schematic illustration of a helical structure of dextrin is also shown in the figure.

charides: For dextrin and dextran sulfate, in particular, a significant delay in the migration time of an analyte, i.e., a decrease of the velocity of the electroosmotic flow (EOF) was observed, probably due to the adsorption of these polysaccharides onto the capillary walls. Usually the capillary is washed with a solution of KOH or NaOH after each run, to obtain reproducible migration times.

3. Ionic polysaccharides

3.1. Heparin

Heparin was first employed for CE enantiomer separation by Stalcup and Agyei [25]. Heparin is a naturally occurring mucopolysaccharide that is used as the agent of choice in the prevention of throm-

bosis following surgery (anticoagulant). Depending upon the source, the basic subunit of heparin may be either a di-, tetra or hexasaccharide composed of uronic acid and glucosamine residues, linked through α(1→4) linkages [28]. Stalcup et al. [25] employed heparin sodium with a molecular mass of ~10 000 (Scientific Protein Laboratory, Waunakee, WI, USA). Percent S information supplied by the manufacturer, 11%, suggested that there is ~one sulfate group per monosaccharide residue (see Fig. 1). Therefore, heparin has the potential for considerable electrophoretic mobility. To determine the electrophoretic mobility of heparin, Stalcup et al. [25] performed the following experiment: The capillary was filled with running buffer without heparin, the detector reservoir was filled with buffer containing heparin, voltage was applied and the change in current was monitored. In this experiment, a rise in current was monitored in buffer at pH 4.5, although there was no rise in current at pH 5.0. This indicates that anodic migration of heparin takes place at pH 4.5. At pH 5.0, the net migration of heparin, i.e., the electrophoretic mobility of heparin was balanced out by the electroosmotic mobility.

The results for seven antimalarial drugs, eight antihistamines and seven other compounds are summarized in Table 3. The buffer solutions employed were 10 m*M* Na_2HPO_4–H_3PO_4 (pH values of 4.5 or 5.0) containing 2% heparin (M_r~10 000). Fifteen of the 22 compounds were successfully enantioseparated under the conditions. By using buffer at pH 4.5, the enantioselectivity (resolution, R_s) of some of the analytes that were partially resolved at pH 5.0 was improved, with a long analysis time. From these investigation (unsuccessful enantiomer separation of quinacrine and halofantrine), they concluded that, in addition to electrostatic interactions, solute size may also play a role in the enantiorecognition of heparin.

They further investigated enantiomer separation by CE using heparin [29]. Chloroquine and chlorpheniramine were selected as analytes and the parameters that affect enantiomer separation, i.e., buffer pH (4.5–8.2), heparin concentration (0.5–5%) and buffer concentration (10–50 m*M*), were examined. Changes in pH affect the migration times of the analytes. A decrease in the pH leads to an increase in the net charge on the basic analytes, therefore, leading to stronger ionic interactions with heparin, resulting in longer migration times. A reduction in the velocity of EOF at low pH also contributes to the increase in the migration times of analytes. Enantiomers of chloroquine, which migrated within 15 min at pH values above 6.9, migrated at 53 and 59 min with R_s=2.97 when a buffer solution of pH 5.0 containing 2% heparin was used. Chloroquine did not elute at pH 4.5. On the other hand, enantiomers of chlorpheniramine were separated at pH 4.5 with migration times of 37 and 41 min.

The concentration of heparin is another important parameter affecting enantiomer separation. In the concentration range investigated, 0.5–4.0%, migration times and R_s values of the enantiomers of chlorpheniramine and chloroquine increased with increasing heparin concentration [29]. Although the increase in the ionic strength and viscosity due the addition of heparin leads to a decrease in the velocity of EOF, the decrease in the migration times can be interpreted mainly by the ionic interactions that took place. According to the Wren and Rowe model for neutral CDs [30,31], there is a maximum value in plots of the R_s vs. the concentration of selector. This is true for ionic selectors. In heparin, the R_s for the analytes mentioned above gradually reached a maximum value. However, a further increase in the concentration of heparin was limited by the increase in current.

Buffer concentration also influenced enantiomer separation. The effect of the concentration of phosphate buffer on migration times for chloroquine was investigated in the 10–50 m*M* range [29]. The migration time of chloroquine decreased with increasing buffer concentration, which is somewhat surprising. Usually, an increase in buffer concentration leads to a decrease in the velocity of EOF and, hence, to an increase in the migration time. The reason for the results found was not clear. It is possible that the phosphate ions either ‘mask’ or compete with the heparin for association with the analytes or that the phosphate may be binding to the wall.

Heparin was also used for the enantiomer separation of oxamniquine, which is an antischistosmiasis drug [32]. Excellent separation (R_s>3) of enantiomers of oxamniquine was achieved with 3 m*M* heparin in 50 m*M* phosphate buffer, pH 3.0. Heparin is a linear polysaccharide linked through α(1→4)

Table 3
Enantiomer separation by CE with ionic or chargeable polysaccharides

Polysaccharide	Enantioseparated analytes	Conditions	Ref.
Heparin	Chloroquine, hydroxychloroquine primaquine, mefloquine, enpiroline pheniramine, chlorpheniramine brompheniramine, carbinoxamine doxylamine, dimethindene tetramisole, tryptophan methyl ester anabasine, nornicotine	10 m*M* phosphate buffer, pH 4.5 or 5.0, 2% C.S.[a] 52.4 cm×75 μm	[25]
	Oxamniquine	50 m*M* phosphate buffer, pH 3.0, 3 m*M* C.S. 50.0 cm×50 μm	[32]
	Diltiazem, 6-chlorodiltiazem 9-chlorodiltiazem, clentiazem trimetoquinol, chlorpheniramine	20 m*M* phosphate–borate buffer, pH 6.0 or 6.5, 3% C.S. 40–50 cm×75 μm	[26]
Chondroitin sulfate C	Diltiazem, 6-chlorodiltiazem 9-chlorodiltiazem, clentiazem trimetoquinol, trimetoquinol isomer laudanosine, laudanosoline norlaudanosine, primaquine propranolol, sulconazole, verapamil	20 m*M* phosphate–borate buffer, pH 2.8–7.0, 3% C.S. 40–50 cm×75 μm	[26,34,37]
Chondroitin sulfate A	Diltiazem, clentiazem, trimetoquinol, trimetoquinol isomer sulconazole, primaquine, verapamil	20 m*M* phosphate–borate buffer, pH 2.5 or 2.9, 3% C.S. 40 cm×75 μm	[37]
Dextran sulfate	Trimetoquinol, trimetoquinol isomer laudanosine, laudanosoline norlaudanosine	25 m*M* phosphate–borate buffer, pH 5.5–7.0, 3% C.S. 50 cm×75 μm	[38]
	Diltiazem, clentiazem, laudanosine	20 m*M* phosphate–borate buffer, pH 6.0 or 6.5, 3% C.S. 40–50 cm×75 μm	[26]
	Chloroquine, chlorpheniramine	10 m*M* phosphate buffer, pH 4.5 or 5.0, 2% C.S. 52.4 cm×75 μm	[29]
λ-Carrageenan	Propranolol, tryptophanol pindolol, laudanosine laudanosoline	25 m*M* citric acid–29 m*M* Tris, pH 4.0, 0.28% C.S. 90 cm×100 μm	[39]
DEAE–dextran	BNC diltiazem synthetic intermediate clentiazem synthetic intermediate	20 m*M* phosphate–borate buffer, pH 8.7, 3% C.S. 40 cm×50 μm	[40]
CM–amylose	Diltiazem	50 m*M* phosphate buffer, pH 3.0, 1% C.S. 47.0 cm×50 μm	[41]

[a] C.S. = chiral selector.

linkages. Therefore, a helical structure may be possible to some extent [33], leading to effective enantioseparation. They used two heparins from different sources, Sigma and Lancaster Synthesis, both of which were extracted from bovine lung tissue and intestinal mucosa of pigs and cattle. They compared the enantioselectivity under the same conditions. As mentioned above, heparin from different sources gave rise to small changes in enantiomer separation. In heparin from Sigma, the migration

times of enantiomers of oxamniquine were 41.7 and 46.3 min, while those in heparin from Lancaster Synthesis were 36.1 and 37.8 min, respectively.

Heparin was successful for the enantiomer separation of diltiazem, its chloro derivatives [26,34] and trimetoquinol [26]. The buffer employed was 3% heparin (from Wako Pure Chemicals) in 20 m*M* phosphate–borate buffer, pH 6.0 or 6.5. Under acidic conditions (pH 3.0), these analytes did not migrate within 50 min, as for other results mentioned above, probably due to the strong ionic interactions and low velocity of EOF.

3.2. Chondroitin sulfate

Chondroitin sulfates are mucopolysaccharides composed of unit structures of β(1→4)- or β(1→3)-linked glucosamine and uronic acid, as shown in Fig. 1. These are natural components found in connective tissue or mast cell granules and are soluble in water, hence, they can be used as chiral selectors in EKC. Nishi et al. [26] first employed chondroitin sulfate C for CE enantiomer separation. They employed three anionic polysaccharides, namely chondroitin sulfate C, heparin and dextran sulfate, for the CE enantiomer separation of basic analytes. Among them, the M_r of chondroitin sulfate C is the largest (30 000–50 000) and its ionic character, i.e., number of anionic groups per monosaccharide unit, is the smallest (one carboxy group or sulfate group per monosaccharide unit), according to the percent S information (7% S) supplied by the manufacture. This means that the electrophoretic mobility of chondroitin sulfate C is the smallest of the three ionic polysaccharides employed.

Preliminary CE enantiomer separation was investigated using buffer solutions with pH values of 3 and 7 containing 3% chondroitin sulfate C. It was found that chondroitin sulfate C could be used under both acidic and neutral conditions, while for the other two (heparin and dextran sulfate), no migration of the basic solutes was observed under acidic conditions. At pH 3.0, most of carboxyl groups probably do not dissociate and only sulfate groups dissociate, because the reported pK_a value of hyaluronic acid, which is a mucopolysaccharide with a similar structure and only a carboxy group as the chargeable residue, is 3.04 [35]. Successful enantiomer separation of diltiazem analogues, trimetoquinol analogues, primaquine, etc. (a total of thirteen of 24 compounds) was achieved, as summarized in Table 3. Among the successful CE enantiomer separations of trimetoquinol, EKC with chondroitin sulfate C gave the largest R_s value. Moreover, all of the enantiomers of trimetoquinol-related compounds (norlaudanosoline, laudanosoline, laudanosine, trimetoquinol isomer) were successfully separated in a single run. The resolution, R_s (ca. 3.6) of diltiazem (*SS*- and *RR*-forms) by EKC with chondroitin sulfate C (pH 2.4, 3% chiral selector) was also the largest among the successful CE enantiomer separation methods used for this drug, such as micellar electrokinetic chromatography (MEKC) with sodium taurodeoxycholate [36]. The four possible optical isomers of diltiazem were successfully separated by EKC with chondroitin sulfate C, as shown in Fig. 3. Although heparin was successful for the enantiomer separation of diltiazem analogues as mentioned above [34], it was not successful at separating each compound, i.e., diltiazem and its chloro derivatives. The most important and useful character of chondroitin sulfate C must be its small ionic character, which permits EKC analysis under acidic conditions, leading to large enantioselectivity.

The effects of buffer pH on migration times and enantioseparation were investigated for trimetoquinol, using 20 m*M* phosphate–borate buffer, pH 3–7, and containing 3% chondroitin sulfate C. As for heparin, the migration times of enantiomers of trimetoquinol increased with decreasing pH values, due to a decrease in the velocity of EOF. Separation of the enantiomers was achieved at all pH values investigated. At pH values of 6.0 and 7.0, migration of the enantiomers of trimetoquinol was slower than that of EOF. In contrast, the enantiomers migrated faster than EOF in the acidic buffers (pH 3–5). Resolution was improved by decreasing the pH. The resolution was 2.8 at pH 3.0, whereas, at pH 7.0, it was 0.5. Additionally, selectivity in the separation not only between the enantiomers but also among the analytes was affected by the pH. The effects of buffer pH on the migration times and resolution were also examined for diltiazem, using the same conditions as for trimetoquinol. The results were almost the same as for trimetoquinol, except that the migration of diltiazem was faster than EOF at all pH

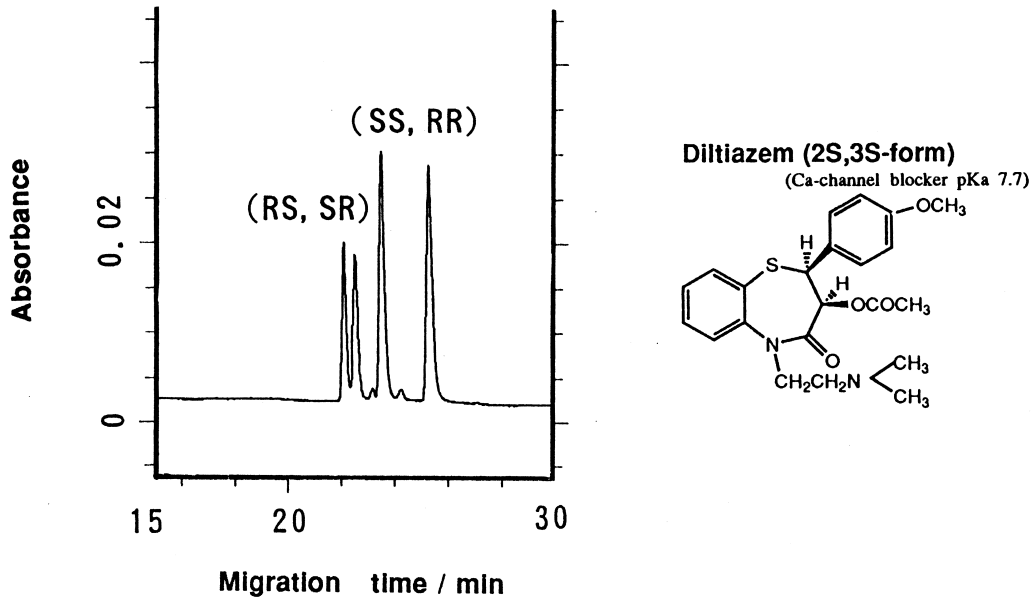

Fig. 3. Separation of enantiomers of diltiazem by EKC with chondroitin sulfate C. Diltiazem is in the *SS*-form. Conditions: 3% Chondroitin sulfate C in 20 m*M* phosphate–borate buffer (pH 2.4); separation tube, 75 μm×50 cm effective length; applied voltage, 20 kV; detection, 235 nm; temperature, room temperature (From ref. [26]).

values. Enantiomers of diltiazem were separated in the pH range investigated. However, acidic conditions were superior to basic conditions because of the slow velocity of EOF. In EKC with chondroitin sulfate C, the pH affected the migration times and the enantioseparation as we had expected, although it was not so critical as in EKC with heparin and dextran sulfate. It is recommended that the EKC enantiomeric separation of basic drugs with the chondroitin sulfate C system be performed under acidic conditions.

The effects of the concentration of chondroitin sulfate C on migration times and enantioseparation were investigated for diltiazem and trimetoquinol using a phosphate buffer solution of pH 2.8 [26]. The concentration range of chondroitin sulfate C tested was from 0 to 3%. By increasing the concentration of chondroitin sulfate C, migration times of the analytes gradually increased and separations of the enantiomers were improved. A marked improvement in the enantiomer separation was observed at a concentration of between 1.0 and 2.0%, as shown in Fig. 4.

Chondroitin sulfate A and chondroitin sulfate C (both as their sodium salts) were employed for the EKC enantiomer separation of eight basic drugs (diltiazem, clentiazem, trimetoquinol, its isomer, verapamil, sulconazole, propranolol and primaquine)

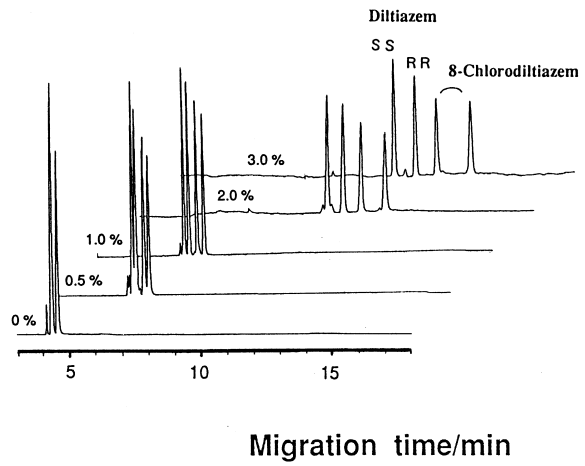

Fig. 4. Effect of the concentration of chondroitin sulfate C on the separation of the enantiomers of diltiazem and clentiazem (8-chlorodiltiazem). Buffer, 20 m*M* phosphate–borate buffer (pH 2.8) containing 0–3.0% chondroitin sulfate C. Other conditions are the same as in Fig. 3. (From ref. [26]).

[37]. A 20 m*M* phosphate buffer solution, pH 2.9, containing 3% of each chondroitin sulfate was used. For the tested compounds, enantiomer separation of propranolol was unsuccessful in chondroitin sulfate A, although all were successfully enantioseparated by EKC with chondroitin sulfate C. A reversal of the migration order was observed for some drugs. Overall, chondroitin sulfate C gave better enantioseparation than chondroitin sulfate A. This may be interpreted from the migration times of the analytes. The migration times of the analytes in chondroitin sulfate A were all shorter than those obtained in chondroitin sulfate C. This can be interpreted by the contribution of the electrostatic interaction between the analytes and chondroitin sulfates, even if this is smaller than that in heparin and dextran sulfate. The structural difference between these two chondroitin sulfates is in the position of the sulfate groups, as shown in Fig. 1. Chondroitin sulfate C is a 6-sulfate and chondroitin sulfate A is a 4-sulfate. Hence there may be some steric hindrance in the 4-sulfate compared with the 6-sulfate. Basic analytes must interact more strongly with the 6-sulfate, leading to longer migration times.

3.3. Dextran sulfate

Dextran sulfate is composed almost exclusively of D-glucopyranose units bonded through α(1→6) linkages. Dextran sulfate exhibits anticoagulant activity, like heparin. The M_r of dextran sulfate is ca. 7300 and the sulfur content is about 17%. This means that there are three sulfate groups per D-glucose unit, as shown in Fig. 1. Dextran sulfate was successfully employed for the enantiomer separation of trimetoquinol and its isomers [38]. For other basic analytes (a total of 24 compounds), five enantiomers were separated [26]. In total, eight of the 28 analytes were enantioseparated by EKC with dextran sulfate. The pH values of the buffers employed were 5–7, because of the strong electrostatic interaction under acidic conditions, as mentioned above for heparin. From the comparison with successful and unsuccessful enantioseparations, analytes with a hydrophobic and bulky group may be essential factors for the enantioseparation in EKC with dextran sulfate. Enantiomer separations of chloroquine and chlorpheniramine using dextran sulfate (M_r=8000, from Sigma) were also reported in comparison with those using heparin [29]. It was found that linear α(1→6)-linked D-glucopyranose polymers also have enantioselectivity, as for other polymers with α(1→4) linkages (i.e., CDs and heparin, etc.).

The effects of the concentration of dextran sulfate on the enantiomer separation and migration times of trimetoquinol and its isomer were investigated [38]. The pH of the buffer solution was 7.0 and the concentration range of dextran sulfate studied was from 0.5 to 5%. Separation of enantiomers of trimetoquinol was not achieved below a concentration of 3%, although the enantiomers of its isomer were separated using more than 2% dextran sulfate. The migration times and resolution gradually increased with an increase in the concentration, as observed for the other ionic polysaccharides mentioned above. However, marked peak tailing was observed with a 5% dextran sulfate solution, compared to the peaks obtained with lower concentrations of dextran sulfate. The reason for this is not clear. The effect of the concentration of dextran sulfate (0–4%) was also investigated for the enantiomer separation of chloroquine and chlorpheniramine [29], using 10 m*M* phosphate buffer, pH 6.0. In contrast to the trimetoquinol and heparin cases, both chloroquine and chlorpheniramine exhibited a maximum R_s value at 3% dextran sulfate, as predicted by the Wren and Rowe equation [30,31].

The effects of buffer pH on the migration times and enantiomer separations of trimetoquinol and its isomer were also investigated, using a 25-m*M* phosphate–borate buffer, pH 3.8–8.4, containing 3% dextran sulfate [38]. The resolution of the enantiomers was improved on decreasing the pH, i.e., through the reduction of the velocity of EOF, although at pH 3.8, no peak was detected within 30 min because of the slower EOF. The same investigation was performed using a 2% heparin solution for the enantiomer separations of chloroquine and chlorpheniramine [29]. In the pH range of 5–8, the same tendency was observed as in trimetoquinol.

The effect of buffer concentration (10–50 m*M*) on dextran sulfate-based separations was investigated for chloroquine and chlorpheniramine [29]. At a lower phosphate buffer concentration (10 m*M*), the velocity of EOF was not independent of the pH (6–8). This suggests that the dextran sulfate may be

adsorbed onto the capillary walls. This may be the reason for the peak tailing mentioned above. In all cases, both chlorpheniramine and chloroquine exhibited longer migration times at the lower phosphate buffer concentration, as was found for heparin [29].

The effects of the M_r of dextran sulfate on migration times and enantiomer separations of chlorpheniramine and chloroquine were investigated, using 10 m*M* phosphate buffer, pH 6.0, containing 2% dextran sulfate [29]. Dextran sulfates with three different M_rs (3100, 8000 and 15 000, from Sigma) were employed. The percentage of S was almost the same for the three dextran sulfates. The migration times of the signal of the EOF and of the analytes, and the current all increased with increasing M_r when the concentration of dextran sulfate was held constant (2.5 m*M*). However, on the basis of equivalent molar concentration, that is, weight %, there was no clear correlation between the M_r and resolution for chlorpheniramine. In contrast, for chloroquine, only dextran sulfate with a M_r of 8000 showed enantioselectivity at that concentration.

3.4. λ-Carrageenan

Carrageenans are water-soluble linear galactan polysaccharides that are extracted from a number of red seaweeds. They are composed of repeated sulfated and non-sulfated galactose and 3,6-anhydrogalactose units, and are joined by alternating α(1→3) and β(1→4) glycosidic linkages (see Fig. 1). There are three major fractions of carrageenans called κ, ι and λ. The primary differences that influence the properties of the three types of carrageenan are the number and position of the sulfate groups on the repeating galactose units. Among the carrageenans, lambda, which has the highest percentage of sulfur (2.7 sulfates per disaccharide subunit), was found to be effective for enantiomer separation [39]. The higher level of sulfate increases the aqueous solubility and produces lower strength gels.

When λ-carrageenan was present, the EOF was found to be relatively stable over the studied pH range (4.0–7.2). However, while resolution of the enantiomers was observed, severe peak tailing also occurred. Acceptable peak shape and resolution of enantiomers were found when a large inner diameter (100 μm) deactivated silica capillary column was used. This may be interpreted as being due to an interaction between λ-carrageenan and the capillary walls (adsorption). The resolution of the analyte, propranolol, was improved on increasing the concentration of λ-carrageenan (0–0.3%), as found for other ionic polysaccharides. However, the use of high concentrations of λ-carrageenan was limited due to the viscosity. An increase in column temperature led to a decrease in the enantiomer separation.

The effect of buffer concentration on the enantioseparation of propranolol was investigated in the range 0–100 m*M*, using a buffer of pH 4.0 containing 0.28% λ-carrageenan. The high buffer concentration decreased the enantiomer separation. This indicates that higher ionic strength in the run buffer may disturb the electrostatic interactions necessary for successful enantioseparation. Among the analytes tested (a total of sixteen), propranolol, pindolol, tryptophanol, laudanosine and laudanosoline were enantioseparated by EKC with citric acid–Tris buffer, pH 4.0 or 7.2, containing 0.28% λ-carrageenan.

3.5. Others

Diethylaminoethyl (DEAE)–dextran (hydrochloride salt), obtained from Fluka (M_r=500 000) was employed for EKC enantiomer separation [40]. On elemental analysis, it was found to contain about 3% nitrogen, which means that there is one DEAE group per two D-glucose units, as shown in Fig. 1. In this case, a coated capillary was used for the separation, to prevent the adsorption of the selector, and acidic analytes were investigated. Of the sixteen compounds investigated using a 20-m*M* phosphate–borate buffer, pH 8.7, containing 3% DEAE–dextran, diltiazem synthetic intermediate, clentiazem synthetic intermediate (both have a carboxyl group in the molecule) and 1,1′-binaphthyl-2,2′-dicarboxylic acid (BNC) were successfully enantioseparated.

Recently, native amylose with various DP values was employed for CE enantiomer separation (mentioned below) [41]. An ionic amylose derivative, carboxymethyl (CM)–amylose (from Sigma) was found to be effective for the enantiomer separation of diltiazem [41]. A 50 m*M* phosphate buffer solution, pH 3.0, containing 1% CM–amylose was used as a buffer.

4. Neutral polysaccharides

4.1. Dextrin

Hydrolysis of starch yields a mixture of amyloses that can further be hydrolysed into shorter oligosaccharides (maltodextrins). Both maltodextrins and CDs consist of D-glucose units that are connected through α(1→4) linkages. Maltodextrins are defined by the FDA as saccharide oligomers that have a DE number of less than twenty. Linear maltodextrins are characterized by DP, corresponding to the number of glucose residues in the molecule. Therefore, maltodextrins of low DE number give a higher average DP value.

D'Hulst and Verbeke [18–21] intensively investigated CZE enantiomer separation by employing maltodextrins. From their investigations with various DE maltodextrins [18], it was found that maltodextrins with low DE values (i.e., high DP values) are better chiral selectors. Various non-steroidal anti-inflammatory drugs (e.g., flurbiprofen, ibuprofen, ketoprofen, etc.) and coumarinic anticoagulant drugs (e.g., warfarin and its analogues) were successfully enantioseparated by CZE with Glucidex 2 (DE=2) [19,20] and Glucidex 6 (DE=6) [21]. The low DE dextrin, Dextrin 10 (DE=10) was also successfully employed for CE enantiomer separation [22,23]. Ionic drugs can be enantioseparated in the dextrin system.

Higher molecular mass dextrins (ca. 3700 to >10 000) than maltodextrins were employed for CZE enantiomer separation [42]. Seventeen of the 41 enantiomers tested were enantioseparated using dextrins, as summarized in Table 4. The separation of the enantiomers of four basic or cationic drugs is shown in Fig. 5, where 20 m*M* phosphate buffer, pH 2.5, containing 6% dextrin (M_r of ca. 3700) was used. The buffer pH values employed were 2.5 for the basic drugs and 7.0 for the acidic drugs. Both basic and acidic drugs were enantioseparated by CZE using dextrins. This relatively wide enantioselectivity of dextrin may be ascribed to the structure of dextrin. It is known that dextrin forms a helical structure and that small molecules, such as iodine, can be included in the hydrophobic interior (iodine color reaction) [43], just as in the cavity of CDs (see Fig. 2). The capacity for inclusion in dextrin, which must be more flexible than that of CDs, may contribute to its relatively wide enantioselectivity. It may be possible to enantioseparate analytes that cannot be separated using the CD system (because of the analyte's bulkiness, i.e., complexation between the analyte and the CDs is insufficient or ineffective) by using the

Table 4
Enantiomer separation by CE with neutral polysaccharides

Polysaccharide	Enantioseparated analytes	Conditions	Ref.
Dextrin	Diltiazem synthetic intermediate clentiazem synthetic intermediate BNP, flurbiprofen, ibuprofen naproxen, warfarin *p*-chlorowarfarin, BNA, diltiazem clentiazem, 6-chlorodiltiazem 9-chlorodiltiazem, sulconazole timepidium, primaquine, verapamil	20 m*M* phosphate–borate buffer, pH 2.5 or 7.0, C.S.[a]= 3–15% 40 cm×75 μm	[42]
Dextran	BNP, BNC, BNA, trimetoquinol trimetoquinol isomer laudanosoline, norlaudanosoline	20 m*M* phosphate–borate buffer, pH 2.5 or 7.0, C.S.=3–15% 40 cm×75 μm	[42]
Amylose	BNP, trihexyphenidyl, Troger's base	100 m*M* phosphoric acid– triethanolamine buffer, pH 3.0 or 7.0, 0.5% C.S.	[41]
MEC, HPC laminaran pullulan	BNP	100 m*M* phosphoric acid– triethanolamine buffer, pH 3.0, 2.0% C.S.	[41]

[a] C.S.=Chiral selector

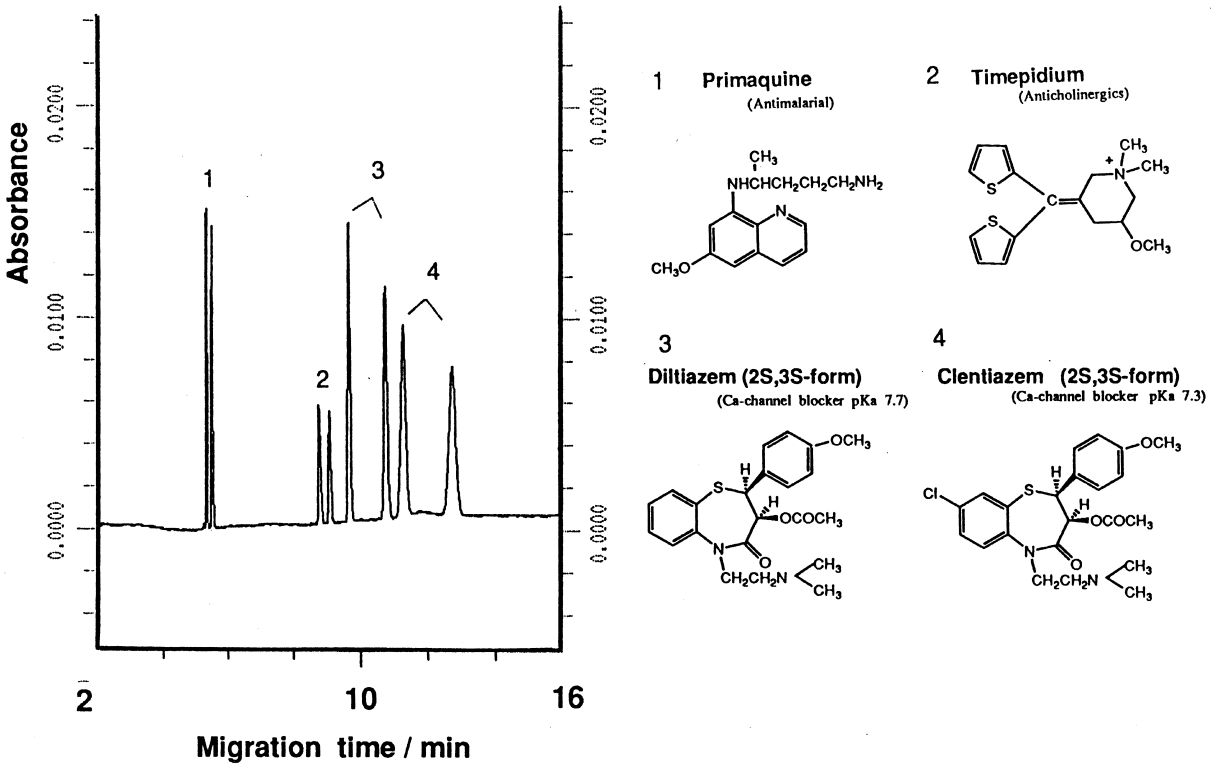

Fig. 5. Separation of enantiomers by CZE with dextrin. Conditions: 15% JP (Japanese pharmacopeia grade) dextrin in 20 m*M* phosphate buffer (pH 2.5); separation tube, 75 μm×40 cm effective length; applied voltage, 30 kV; detection, 220 nm; temperature, 20°C. (From ref. [42]).

dextrin system. Diltiazem, which could not be enantioseparated by CZE with various CDs including charged CDs, was baseline-enantioseparated by CZE with dextrin. Thus, this method was successfully applied to the testing of the optical purity of diltiazem [42]. The minor enantiomer (0.1%; *RR*-form) could be detectable in the major enantiomer (*SS*-form) by this method, as shown in Fig. 6. Other than the separation, there was a significant delay in the velocity of EOF in the dextrin system. Washing the capillary with an alkaline solution was essential for obtaining reproducible migration times in the dextrin system.

The effect of the molecular mass of dextrins on enantioseparation was investigated with 20 m*M* phosphate–borate buffer, pH 7.0, containing 3% of each dextrin (M_rs of 3700, 6600 and >10 000) [42]. The results were compared with those from CZE without dextrin. The α and R_s values obtained for the analyte tested, 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (BNP), were almost the same for the three dextrins.

The effects of the concentration of dextrin on migration times and the enantioseparation of an anionic solute, BNP, were investigated using an uncoated capillary, 20 m*M* phosphate–borate buffer, pH 7.0, containing 0–6% dextrin (M_r=3700) and a positive applied voltage [42]. The migration times and $\mu_{ep(S)-eff}$ ($=\mu_{ep(S)-obs}-\mu_{eo}$) for the analyte are shown in Fig. 7, where $\mu_{ep(S)-obs}$ is the apparent electrophoretic mobility of the analyte and μ_{eo} is the electroosmotic mobility, determined from the migration of mesityl oxide. The minus sign means that the direction is towards the positive end. The effects of the concentration of dextrin (M_r=3700) on migration times and enantioseparation were also investigated for a basic solute, diltiazem, using a 20 m*M* phosphate buffer, pH 2.5 [42]. When dextrin was

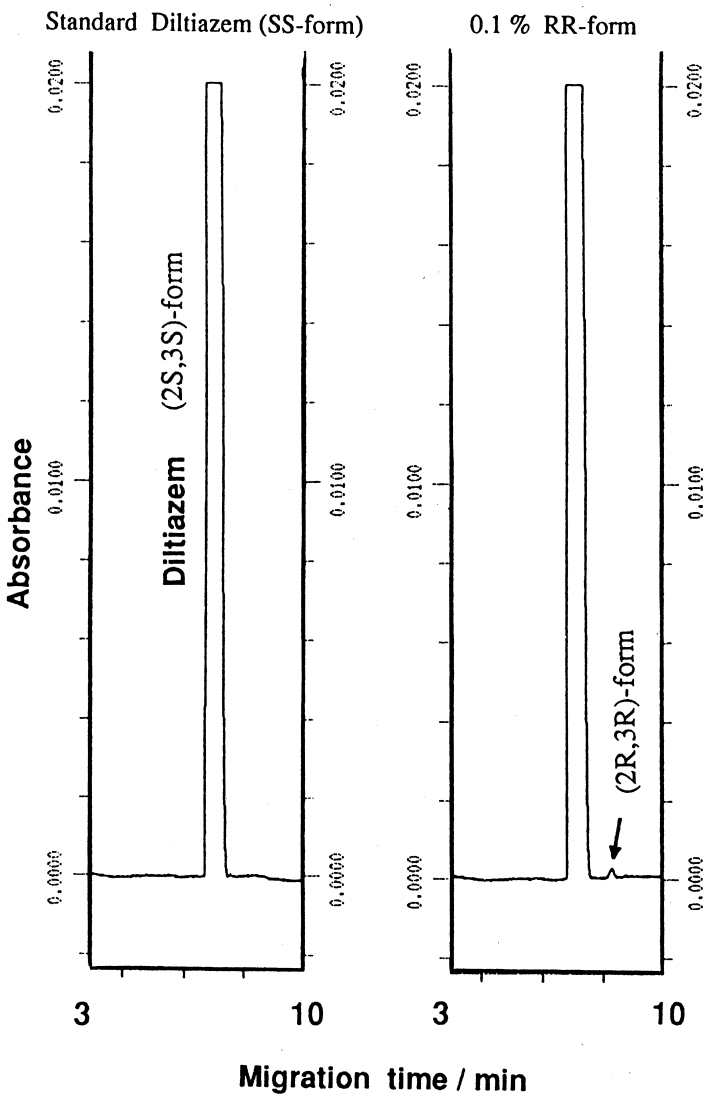

Fig. 6. Testing the enantiomer purity of diltiazem by CZE with dextrin. Conditions: 9% JP dextrin in 20 m*M* phosphate buffer (pH 2.5). Other conditions are the same as in Fig. 5. (From ref. [42]).

used, the migration times of both acidic and basic solutes increased with increasing concentration. The differences between the enantiomers' electrophoretic mobilities were also increased and the R_s values were improved with increasing dextrin concentrations (up to 6%).

Next, the effect of buffer concentration on the enantiomer separation of diltiazem was investigated with 10–50 m*M* phosphate buffer, pH 2.5, containing 3% dextrin ($M_r = 3700$) [42]. Migration times increased with increasing buffer concentration (from 4.5 and 4.7 min at 10 m*M* to 5.5 and 5.8 min at 50 m*M*) and the R_s was improved (from 1.7 at 10 m*M* to 2.5 at 50 m*M*). This can be interpreted as a result of the change in μ_{eo}, which decreases with increasing buffer concentration [44].

Finally, the effect of buffer pH on enantioseparation was investigated for diltiazem using 20 m*M* phosphate buffer solution containing 3% dextrin ($M_r = 3700$) [42]. Baseline enantiomer separation

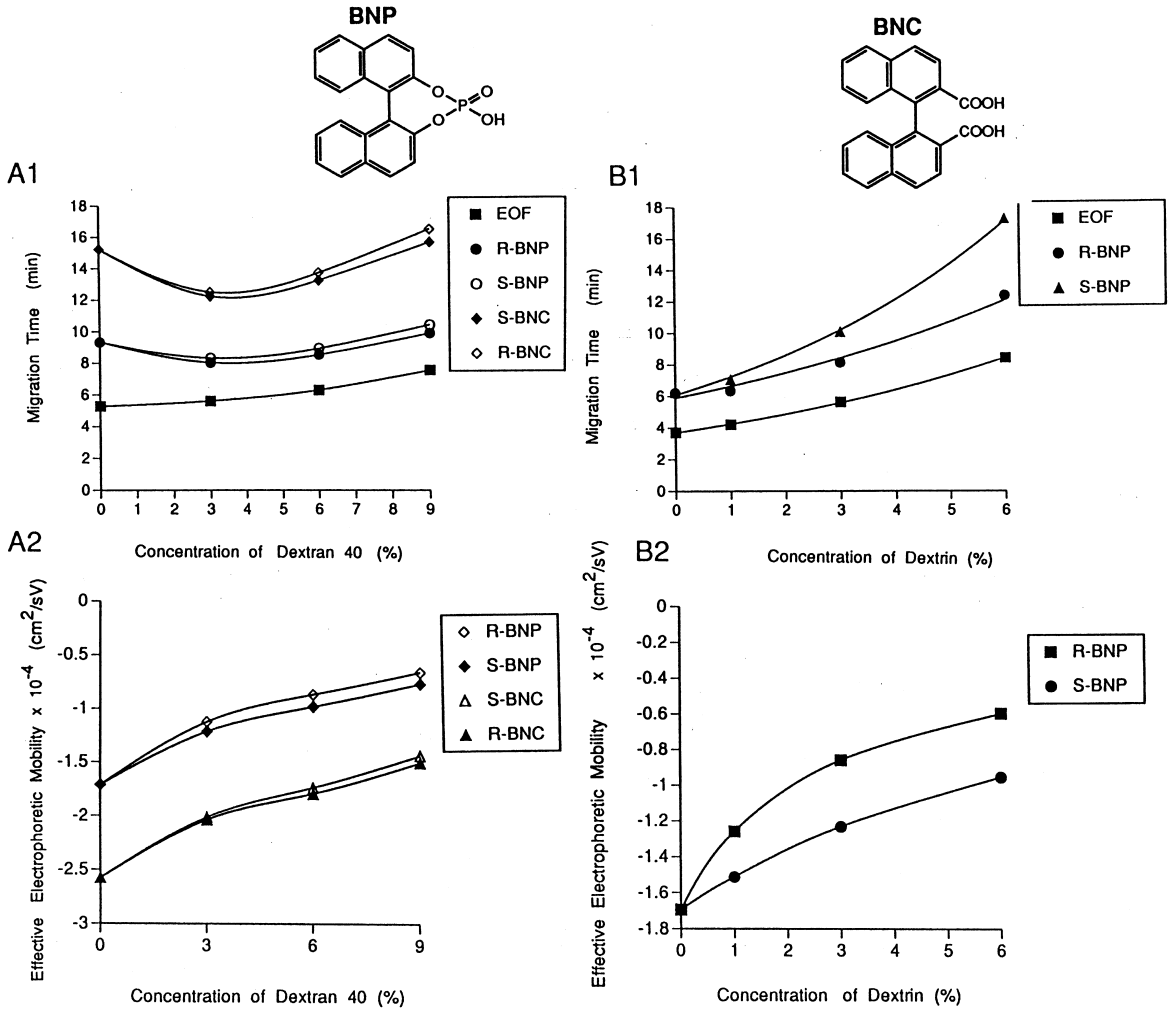

Fig. 7. Effect of the concentrations of dextrin and dextran on the migration times and effective electrophoretic mobilities of BNP and BNC. (A) Dextran 40 and 15 kV, (B) JP dextrin and 20 kV. Buffer pH is 7.0. Other conditions are the same as in Fig. 5, except for the polysaccharide concentrations. (From ref. [41]).

was observed in buffer solutions of pH<4.5. Above pH 5, the strong EOF generated decreased the migration times of the analyte and the R_s was reduced, as in the other CD-modified CZE enantiomer separations of basic drugs.

4.2. Dextran

D'Hulst and Verbeke [18] also investigated CZE enantiomer separation using dextrans, which are mainly linked through α(1→6) bonds. Dextran 1500 (8.2%) and dextran 6000 (10%) were not effective for the enantiomer separations of flurbiprofen, ibuprofen and ketoprofen, which were all successfully enantioseparated by CZE with maltodextrins. Enantiomer separations of flurbiprofen, suprofen, indoprofen, ibuprofen and fenoprofen were not successful by CZE with 10% dextran (M_r ca. 40 000) [22]. From these results, they confirmed the importance of the α(1→4) linkage for enantiomer recognition. Dextran was first employed for CZE enantiomer separation through the bovine serum albumin (BSA)–dextran polymer network [45]. Separation of leucovorin enantiomers was achieved using a coated

capillary filled with a BSA–dextran polymer network. However, in this case, BSA is essential for enantiomer separation.

Nishi et al. [37,42] first reported the successful enantiomer separation by CZE with dextran (M_rs of 40 000–300 000) [37,42]. When dextran was used, the viscosity of the solutions increased with increasing M_r, although the dextrin solutions were of relatively low viscosity [42]. In contrast to the dextrin system, the delay in the velocity of EOF was not so large in the dextran system. This may be due to the adsorption of polysaccharides on the capillary walls. However, this seemed to be dependent on their structure because the molecular mass of dextrin (3700–10 000) is much smaller than that of dextran (40 000–300 000). Among the analytes tested (41 enantiomers), BNP and BNC (acidic analytes), and 1,1′-binaphthyl-2,2′-diamine (BNA), trimetoquinol and its analogues (basic analytes) were enantioseparated with higher concentrations (15% dextran) compared with the concentration employed for dextrins (usually 3–9%). Of the 41 enantiomers that were tested, 22 were enantioseparated by CZE with dextrin or dextran (Table 4). From the investigations with dextran and dextran sulfate mentioned above (see Section 3.3), it was found that polysaccharides that mainly have $\alpha(1\rightarrow6)$ linkages are also useful chiral selectors, although enantioselectivity in dextrans was not as good as in dextrins, as judged by the numbers of enantioseparated analytes.

The effect of the molecular mass of dextran on enantioselectivity was investigated for BNP with 20 m*M* phosphate–borate buffer, pH 7.0, containing 3% dextran (M_r values: ca. 40 000, ca. 70 000, 100 000–200 000, 200 000–300 000). Resolution was slightly improved as the M_r increased in the range 40 000–300 000, although the separation factors and the migration times were not changed. These results corresponded well with those for dextrin [42] and dextran sulfate for chlorpheniramine [29]. These indicate that polysaccharides with a molecular mass above a certain level, ca. 3000–4000, may show the same enantioselectivity at the same percentage concentrations, although there was a relationship between the enantioselectivity and molecular mass in much smaller oligosaccharides, such as maltodextrins [18]. In capillary gel electrophoresis with polymer solutions, it was found that the pore size of the polymer solution is not dependent on the degree of polymerization (expressed as the molecular mass) but only on its concentration [46]. For small molecules such as drugs, these pore sizes may be related to the strength of the interaction between the analyte and the matrix, leading to enantiorecognition of the analytes. Two solutions of the same type of polysaccharide at the same concentration but with different molecular masses (within a certain M_r range) will give the same separation pattern. However, there may be an upper limit of molecular mass where this hypothesis needs to be altered, considering the results by dextran sulfate for chloroquine [29] and amylose mentioned below [41].

The effects of the concentration of dextran (M_r = 40 000) on migration times and the enantioseparation of BNP were investigated using an uncoated capillary and a positive applied voltage in the concentration of range 0–6% [42]. The results are shown in Fig. 7. The migration times of BNP decreased on addition of 3% dextran, although μ_{eo} was delayed as in the dextrin system. This is probably because of the large interaction of BNP with dextran and the relatively small delay in μ_{eo}. The values of $\mu_{ep(S)-eff}$ clearly show an increase of the analyte–polysaccharide interaction with increasing concentration. Dextran and dextrin have no electric charge and migrate with the EOF; consequently, a decrease in $\mu_{ep(S)-eff}$ means an increase in the interaction between anionic analytes and these polysaccharides. Values of R_s and α were improved by increasing the polysaccharide concentration.

4.3. Amylose

Native amylose and cellulose are usually insoluble in water. However, high molecular mass amylose is soluble in water [41]. Various amyloses (Am) with different DP values, namely Am-4900 (DP = 4900), Am-6250 (DP = 6250) and Am-16 250 (DP = 16 250), were employed for CZE enantiomer separation by Chankvetadze et al. [41]. Enantiomer separations of BNP, trihexyphenidyl, Troger's base, mianserine and ibuprofen were achieved using 100 m*M* H_3PO_4–triethanolamine buffer, pH 3.0 or 7.0, containing 0.5% Am-4900.

The effect of DP, that is, the molecular mass on the enantiomer separation was investigated by em-

ploying three amyloses within a narrow molecular mass distribution ($M_w/M_n<1.1$) [41]. The tested analytes were BNP and diltiazem. Sodium dihydrogenphosphate buffer, pH 3.0, containing 0.22% of each amylose was used as the running buffer. In contrast to the results found with dextran in the range 40 000–300 000 [42], dextrins with M_r values of 3700–>10 000 [42] and dextran sulfate (M_r values of 3100–15 000) for chlorpheniramine [29], there was a relationship between the DP and enantioselectivity. The highest DP amylose, Am-16 250, could not separate both enantiomers. The reason is not clear at present.

The effect of the concentration of amylose on the enantiomer separation of BNP and diltiazem was investigated with Am-6250 [41]. The values of α and R_s obtained for the analytes are shown in Fig. 8. Both increased with increasing concentrations of Am-6250 and reached the maxima at about 1.5%, after which, they decreased on increasing the concentration further. These results corresponded well with those derived from the Wren and Rowe model [30,31]. However, decreases in both values at above ca. 1.5% were sharper than those observed in the other chiral additives such as CDs [47,48]. It would be interesting to investigate enantioselectivity using much higher concentrations of Am-6250. However, with Am-6250, using concentrations above ca. 2% was impossible due to its extremely high viscosity.

4.4. Others

Other than dextrins (maltodextrin, dextrin, amylose), all of which consist of α(1→4)-linked D-glucose, natural polysaccharides, laminaran, pullulan, and low viscosity methyl cellulose (MEC) and hydroxypropyl cellulose (HPC) showed enantioselectivity. Enantiomers of BNP were separated under

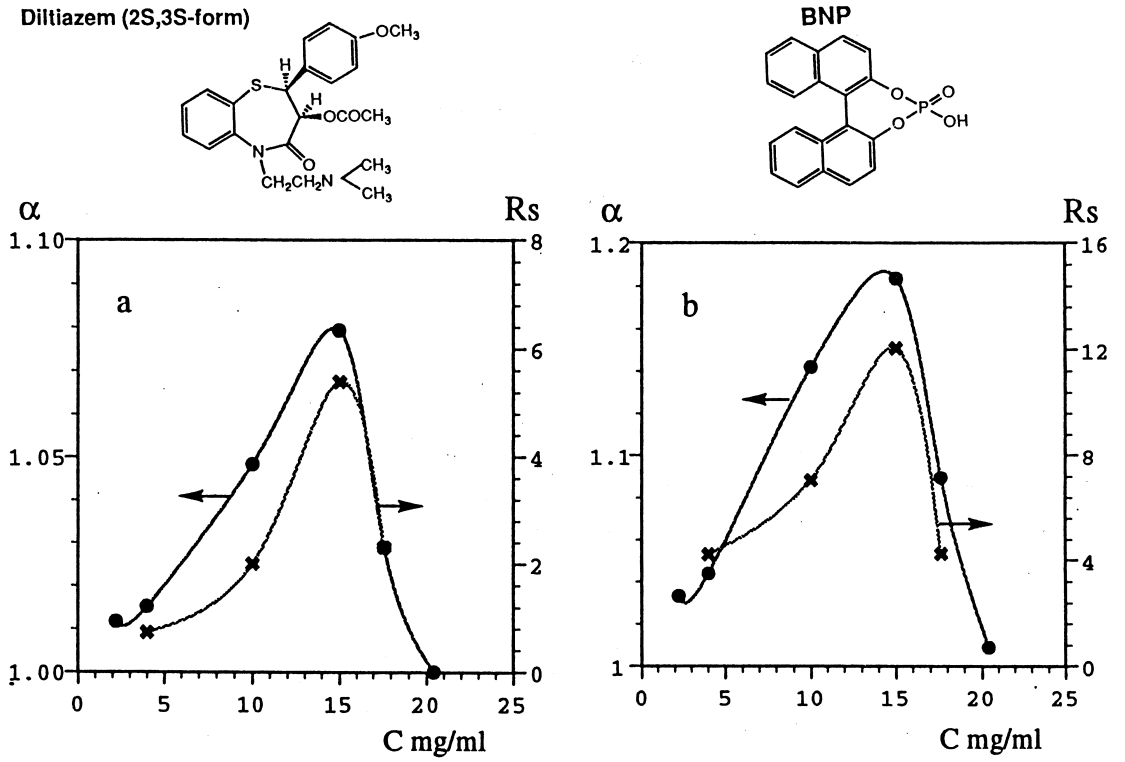

Fig. 8. Effect of the concentration of amylose on the enantioselectivity of diltiazem and BNP. Conditions: Sodium dihydrogen phosphate buffer (pH 3.0) containing selector. Separation tube, 50 μm×48 cm effective length; applied voltage, 20 kV; detection, 210 nm. (From ref. [41]).

acidic conditions (pH 3.0) with 2% solution of MEC, HPC, pulluran or laminaran [41].

5. Cyclodextrin polymers

5.1. Carboxymethyl β-cyclodextrin polymer

Enantiomer separation was also successful using CD polymers, which can be regarded as a type of polysaccharide. In CE enantiomer separation, two CD polymers, namely carboxymethyl (CM)–β-CD polymer [49] and neutral β-CD polymer [50–53], have been employed. According to the manufacturer (Cyclolab), the molecular mass of the CM–β-CD polymer is 6000–8000 and it is soluble in water >20%. β-CD is cross-linked by 1-chloro-2,3-epoxypropane. The estimated number of carboxylic acids per CD ring is two.

In the CM–β-CD polymer, the CD could be used in either a charged or an uncharged state, by selecting the pH of the buffer. Therefore, the effect of the pH of the buffer on the effective mobility of basic analytes was investigated in the range 2.6–6.2 [49]. As expected, at pH 4.5, stronger interaction was observed than at lower pH values. For example, propranolol and terbutaline, which migrated before the EOF signal at pH 2.5, migrated after the EOF signal at pH 4.5. Among the analytes investigated, norphenylephrine, terbutaline, ephedrine, ketamine, propranolol, etc., were successfully enantioseparated by CE with CM–β-CD. The effect of the concentration of CM–β-CD (up to 2%) on enantiomer separation was investigated [49]. The resolution was improved by increasing the concentration ($R_s = 1$ at 1% and 2.4 at 2% for epinephrine).

5.2. Neutral β-cyclodextrin polymer

Neutral β-CD polymer is synthesized by condensation of β-CD molecules with epichlorohydrin ($M_r = 3000–5000$, from Cyclolab). A neutral β-CD polymer solution of up to 10% can be prepared. In neutral β-CD polymer, the separation mode is CZE and only charged enantiomers can be separated. Nishi et al. [50] investigated the enantiomer separation of basic drugs, such as trimetoquinol and its analogues, with β-CD and neutral β-CD polymer (from Wako) under acidic conditions. On comparing the results for three analytes (trimetoquinol, laudanosoline and norlaudanosoline), higher resolution and α values were obtained for the polymer type of β-CD. This may be ascribed to the structure of the polymer. A decrease in the free rotation of the β-CD unit, a constant distribution of β-CD units or hydrophobic interactions, hydrogen bonding, etc., in the polymer network of the neutral β-CD polymer probably contribute to the enantiorecognition.

The effect of the concentration of the neutral β-CD polymer on enantiomer separation was also investigated in the range of 1–7%. The resolution and α values improved on increasing the concentration of the polymer. The addition of sodium dodecyl sulfate to the neutral β-CD polymer solution was also investigated and found to be useful for improving of the separation.

Neutral β-CD polymer was successful for the enantiomer separation of many adrenergic agonists, β-blockers, anaesthetics, etc. [51]. The effect of the addition of organic solvent on enantioselectivity was extensively investigated by Ingelse et al. [52] and Sevcik et al. [53].

6. Enantiorecognition by polysaccharides

Polysaccharides in solution freely rotate or move. Enantiorecognition of polysaccharides in free solution is still not clear, however, as in the previous investigations by HPLC with polysaccharide chiral stationary phases [17], hydrophobic interaction, hydrogen bonding, dipole–dipole interactions, etc., must be important. Some macroscopic structure (helical) or polymer network will also contribute to enantiorecognition. Kano et al. [54] investigated the mechanisms of molecular complex formation of linear α(1→4)-linked oligosaccharides using maltodextrin (G_n, $n = 2–7$) and BNC. With the exception of maltopentaose (G_5), enantiorecognition of BNC was achieved by CZE as shown in Fig. 9. A 40 m*M* carbonate buffer (pH 9–9.5) containing 0.4 *M* G_n was used as the running buffer. In G_2 (maltose), G_3 (maltotriose) and G_4 (maltotetraose), migration times of the *S*-enantiomer were shorter than those of the *R*-enantiomer. Partial separation ($\alpha = 1.02$) was achieved by G_2 and baseline enantioseparation by G_3

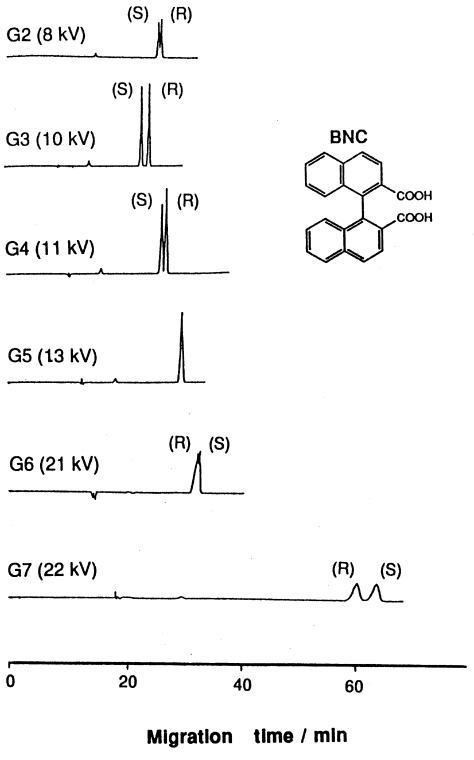

Fig. 9. Enantiomer separation of BNC by CZE with linear maltodextrin. Conditions: 40 m*M* carbonate buffer (pH 9–9.5) containing 0.4 *M* G_n. (From ref. [54]).

($\alpha = 1.10$) was observed. However, in G_4, α decreased (1.09) and no enantioseparation occurred in the case of G_5. Then, enantiomers of BNC were separated again by G_6 ($\alpha = 1.03$) and G_7 ($\alpha = 1.08$) with the enantiomers migrating in the reverse order. From these investigation, hydrogen bonding as well as hydrophobic interactions was suggested as an essential force for enantioselective complexation between saccharide and the anionic binaphthyl analyte, BNC. In the case of G_2 or G_3, which did not have helical structures like amylose [43], hydrogen bonding in the microscopic structure of the saccharide molecule seems to be essential for enantiorecognition. However as can be expected, a helical structure becomes remarkable as increasing the number (*n*) of glucose residues from the molecular mechanics–molecular dynamics calculations. In G_6 and G_7, the helical and the hydrophobic environment is constructed and hydrophobic interaction becomes essential for enantiorecognition. For the neutral polysaccharides having $\alpha(1\rightarrow4)$ linkages, such as dextrin (amylose), these interactions will occur and play an important role in enantiorecognition. In other polysaccharides that are linked by $\alpha(1\rightarrow6)$ bonds and cannot form a helical structure, the polymer network (pore size) will affect enantiorecognition. Furthermore, in ionic polysaccharides, electrostatic interaction plays an important role in enantiorecognition, as mentioned above.

7. Conclusions

CE enantiomer separation, including EKC and CZE, has many advantages over HPLC enantiomer separation as mentioned above. It is usually easy to find successful conditions for enantiomer separation of the analytes being investigated. Most enantiomers have been separated by applying one of several CE modes. CE chiral method development kits are now commercially available from several sources. CDs are used as the starting chiral selectors in the kits because of their wide enantioselectivity.

Recently, oligosaccharides and polysaccharides have been found to be effective as chiral selectors in CE. In particular, mucopolysaccharides such as heparin and chondroitin sulfates, which are natural ionic polysaccharides, and dextrins showed a wide enantioselectivity. Although the enantiorecognition mechanism of these polysaccharides is still not clear, these are the starting candidates for chiral selection that are used in method development for the enantiomer separation of an analyte. We can say that buffer pH and the selector concentration are two of the most important parameters affecting enantioseparation. It is also noted that natural polysaccharides from different sources may give different enantioselectivities because of the molecular mass distribution and the different ratios of the unit components or of the bonding species in the mixture.

For ionic analytes, dextrins should be included in one of the starting chiral selectors. Ionic analytes that could not be enantioseparated by CE with the typical CDs because of their bulkiness or their lack of fit into the CD cavity may be enantioseparated by the flexible helical structure of dextrin, as was found in the diltiazem case. Chondroitin sulfate C will be another candidate for the CE chiral selection of basic

Table 5
Recommended agents as the starting chiral selectors

Analytes	Chiral selectors
Ionic compounds (cationic and anionic)	CDs dextrin
Basic compounds	CDs dextrin chondroitin sulfate C heparin
Neutral compounds	Charged CDs heparin

or cationic analytes, judging from the successful enantiomer separations of trimetoquinol analogues. Relatively weak electrostatic interactions as well as the structure of the polymer of chondroitin sulfate C play important roles in enantiorecognition. Furthermore, chondroitin sulfate C can be used under acidic conditions, which are favourable for the CE enantiomer separation of basic compounds. Heparin will also be useful for the enantiomer separation of basic and neutral analytes because of the electrostatic interactions and the helical structure of the polymer. The recommended starting chiral selectors, including CDs, are summarized in Table 5.

Although the reproducibilities of the migration times and peak areas and the sensitivity of UV detectors are inferior to those given by fully automated modern HPLC instruments, the performance of CE enantiomer separation is satisfactory for the testing of the optical purity of drugs. Quite recently, new CE assay methods were incorporated into monographs of the United States Pharmacopeia [55,56]. One of these methods is for assaying an epinephryl borate ophthalmic solution, where 2,6-di-*O*-methyl-β-CD is used for the separation of (−)- and (+)-epinephrine. Furthermore, in CE, novel chiral selectors will be easier to use. There is a strong possibility of developing specially designed selectors that have wide enantioselectivity. Studies on the chiral discrimination of the CDs employed for the CE enantiomer separation [57–59] and polysaccharide-type chiral stationary phases for HPLC [17,60] by ^{1}H-NMR and ^{13}C-NMR will give some information for designing novel polysaccharide-type CE chiral selectors.

8. Abbreviations

Am	Amylose
BNA	1,1′-Binaphthyl-2,2′-diamine
BNC	1,1′-Binaphthyl-2,2′-dicarboxylic acid
BNP	1,1′-Binaphthyl-2,2′-diyl hydrogen phosphate
BSA	Bovine serum albumin
CD(s)	Cyclodextrin(s)
CE	Capillary electrophoresis
CM	Carboxymethyl
CZE	Capillary zone electrophoresis
DE	Dextrose equivalent number
DEAE	Diethylaminoethyl
DP	Degree of polymerization
EKC	Electrokinetic chromatography
EOF	Electroosmotic flow
G_n	Maltodextrin
G_2	Maltose
G_3	Maltotriose
G_4	Maltotetraose
G_5	Maltopentaose
G_6	Maltohexaose
G_7	Maltoheptaose
HPC	Hydroxypropyl cellulose
HPLC	High-performance liquid chromatography
MEC	Methyl cellulose
NMR	Nuclear magnetic resonance

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