Charged Cyclodextrin Derivatives as Chiral Selectors in Capillary Electrophoresis

B. Chankvetadze,† G. Endresz and G. Blaschke

Institute of Pharmaceutical Chemistry, University of Münster, Hittorfstr. 58–62, 48149 Münster, Germany

1 Introduction

Dramatic differences have been observed in the biological action of enantiomers, which are especially important for pharmaceuticals, food additives and agrochemicals. Regulatory issues are in preparation in many countries which require pharmaceutical companies to consider enantiomers as different entities and to test their pharmacological, pharmacokinetic and toxicological properties separately.[1]

Thorough studies of stereochemical effects in drug action require a cost-effective, easy to handle and versatile technique for enantiomeric analysis. Almost all instrumental separation techniques such as gas chromatography (GC), high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC) and capillary electrophoresis (CE) have been used extensively for enantiomeric analysis during the last decade. These techniques are considered to be complementary. For example, capillary GC offers high efficiency but it is only useful for volatile compounds. HPLC is applicable for nonvolatile compounds but it has lower efficiency than GC. SFC was anticipated to combine the advantages of GC and HPLC (the high efficiency of GC and the possibility to perform liquid phase analyses like HPLC). A number of successful applications of SFC for the separation of enantiomers have been published, but some conceptual and instrumental problems limit the routine application of this technique. CE, which is a free-solution microseparation technique, meets many requirements of modern analysis and CE equipment is available from several instrumental suppliers.

Although the basic principles of electrophoretic phenomena have been known since the nineteenth century, CE has been developed as an instrumental microanalytical separation technique between the mid-60s and the early 80s in pioneering work by Hjertén,[2] Virtanen,[3] Everaerts *et al.*[4] and especially by Jorgenson and Lukacs.[5]

The first separation of enantiomers by CE was reported ten years ago by Zare and coworkers.[6] The field of chiral CE remained in relative obscurity until the appearance of two key papers in 1989. Fanali demonstrated the potential of natural macrocycles, cyclodextrins (CD), as chiral selectors in free-solution CE,[7] and Terabe showed the first application of a charged CD as a potential chiral selector in capillary electrokinetic chromatography (CEKC).[8] Native and neutral derivatized CDs have been employed very extensively as chiral selectors ever since. In contrast, a four-year standstill was observed in application of charged CDs, which is presently receiving a great deal of attention. What are the challenges of CE and especially of CE using charged CDs for enantioseparation ?

(1) From the conceptual point of view, CE is an orthogonal technique to HPLC, GC and SFC, which are based on the partition of analytes into two (mobile and stationary) phases.

(2) CE is a more versatile technique than all the other above mentioned techniques; it takes less than a minute to change one separation medium and/or selector with another. This change is undesirable, laborious and time consuming in other separation based techniques.

(3) CE is a cost-effective technique; unlike other techniques the separation does not need a special column, is commonly performed in aqueous medium, buffered with inorganic salts, and consumes very small amounts of a chiral selector.

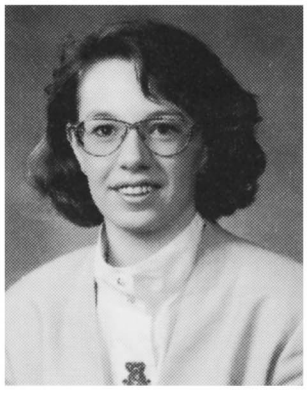
Another important advantage of CE (other than enantioseparation), which is essential for chemistry, biochemistry and biomedicine in general, is that this technique operates in free solution. Therefore, the selector–solute interactions in CE mimic the receptor–ligand interaction in solution, as well as in biological systems, much better than in other techniques which use immobilized selectors.

† *Permanent address*: Department of Chemistry, Tbilisi State University, Chavchavadze ave 1, 380028, Tbilisi, Georgia

Bezhan Chankvetadze was born in Georgia (former USSR) in 1957. He obtained his diploma in physical chemistry at the University of Tbilisi, Georgia, in 1979 and his PhD at the Institute of Organic Chemistry of USSR Academy of Science, Moscow, in 1985. In 1985 he was appointed as Associate Professor at the Department of Physical Chemistry, School of Chemistry, Tbilisi State University in Georgia. He has held research positions with Prof. G. Blaschke at the University of Munster, Germany, in 1991, and 1993–1995 and in the laboratory directed by Prof. Y. Okamoto at Nagoya University, Japan (1992–1993). At present he is continuing his research at Nagoya University as a JSPS fellow. He has published more than 60 research papers in the areas of GC, HPLC and CE. He has coauthored one book, several book chapters and holds several patents. His current research interests include enantiomeric separations by HPLC and CE and study of chiral recognition mechanisms.

Gabriele Endresz was born in Hofgeismar, Germany in 1968. After graduation from the high school in Kassel in 1987 she studied pharmacy at the University of Braunschweig, from which she graduated in 1992. In 1991–92 she spent two years of practical training in a public pharmacy and in the Analytical Research Department at Boehringer Ingelheim KG. Since 1993 she has been working for a PhD in the research group of Prof. G. Blaschke at the Institute of Pharmaceutical Chemistry, University of Munster.

B. Chankvetadze



G. Endresz

G. Blaschke

Gottfried Blaschke was born in Loschitz close to Vienna, Austria, in 1937. He obtained his diploma and PhD in Chemistry from the University of Gottingen in 1962 and 1964, respectively. After post-doctoral research at the University of California, Berkeley, with Prof. H. Rapoport he joined the Institute of Pharmaceutical Chemistry in Kiel, Germany, in 1967 and was appointed Assistant and Professor at the University of Bonn and has been Full Professor at the University of Munster since 1980. His research activities have focused on the analysis of drugs and drug metabolites in biological fluids including chiral separations, capillary electrophoresis and laser induced fluorescence detection.

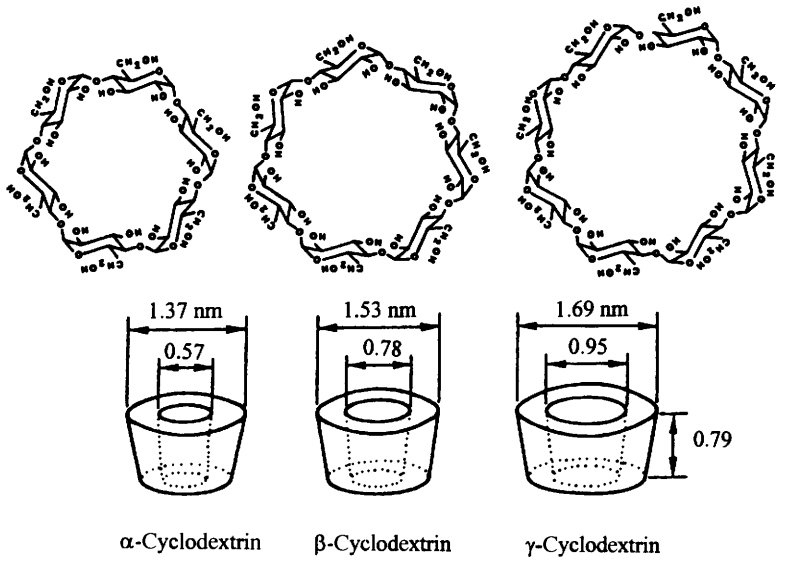


Figure 1 Molecular dimensions (*d*/nm) and structures of α-, β- and γ-CD.

A disadvantage of CE in free solution however is that it is applicable only to charged compounds. The separation in CE is based on the difference in the effective electrophoretic mobilities of the analytes. Neutral substances that lack electrophoretic mobility cannot be resolved using convenient CE techniques. Since the introduction of the chromatographic separation principle in CE by Terabe and coworkers in 1985, this technique has been utilized for the separation of neutral compounds.[9] The last technique, called capillary electrokinetic chromatography (CEKC), operates in two (pseudo)phases with different mobilities. To fulfil this condition the additive of the buffer solution should possess self-mobility. Charged CDs meet this requirement, unlike neutral ones, which makes them useful as chiral selectors for the enantioseparation of neutral racemates. This, and a number of other advantages of charged CDs as chiral selectors in CE, are summarized in this review.

2 Cyclodextrins and their Properties

Cyclodextrins are cyclic oligosaccharides prepared by hydrolytic degradation of starch using the microorganism *Bacillus macerans*. They were discovered in 1891 by Villiers.[10] Today α-, β- and γ-CDs which consist of 6, 7 and 8 D-glucose units respectively, linked by α-1,4 bonds, are commercially available. The number and binding mode of D-glucose units determine the shape and size of CD molecules (Fig. 1). The wider side of the truncated CD cone is formed by the secondary 2- and 3-hydroxy groups and the narrow side by the primary 6-hydroxy groups. Thus, the location of the hydrophilic hydroxy groups on the outer CD rims determines their solubility in water. The inner rim of the CD cavity is lined by hydrogen atoms and the glycosidic oxygen bridges. The nonbonding electron pairs of the glycosidic oxygen bridges are directed towards the inside of the cavity. As a result, the CD cavity is relatively hydrophobic. Intramolecular hydrogen bonds formed between the 2-hydroxy and the 3-hydroxy groups of adjacent glucose units maintain the remarkably rigid structure of CDs. Additionally, each D-glucose unit in the CD structure contains five chiral carbon atoms and as a result the CD macrocycle is chiral. All these properties (solubility in aqueous media, hydrophobicity of the cavity, rigid structure and chirality) are of crucial importance for the application of CDs.

The most remarkable property of CDs, their ability to act stereoselectively by complexation, was discovered by Cramer in 1952:[11] '. . . Cyclodextrins differentiate not only molecules with different shape but also optical antipodes too . . .'. This statement was confirmed by the optical enrichment of racemic aromatic hydroxy acid esters *via* complexation with β-CD.[11]

CDs are now in use in the pharmaceutical industry as solubilizers, diluents or as tablet ingredients which improve the stability, bioavailability and pharmacokinetic properties of drugs. In the food, cosmetic, toiletry and tobacco industries CDs are used either for the stabilization of flavours and fragrances or for the elimination of undesired tastes. In the chemical industry CDs are used as catalysts or catalyst additives, and as separation media for some industrial-scale products. The ability of CDs to bind stereoselectively chiral organic molecules led to widespread applications in analytical sciences and especially for enantioseparations in GC, HPLC, SFC and CE.[12] A number of papers relating to the application of CDs and their derivatives as chiral shift reagents in NMR spectroscopy have been published.

3 Charged CD Derivatives and their Characteristics

The hydroxy groups on both the primary and the secondary sides of the CD rim are the most common reaction sites and have been extensively derivatized.[13–17]

The primary purpose of the chemical modification of CDs was the introduction of:

(1) specific binding and catalytic sites to mimic enzymes;[15]
(2) short alkyl or hydroxyalkyl substituents to modify the solubility and cavity dimensions[13] or, alternatively, introduction of a long alkyl chains with ionic end groups to give the parent compounds micellar properties;[13]
(3) ionic substituents directly on the CD rim or connected with it *via* a short alkyl chain in order to enhance the solubility in aqueous media and improve the biocompatibility of CD derivatives.[13,14]

This last type of CD derivatives is commonly used as chiral selectors in CE and will be discussed in detail in this section.

3.1 Cationic Derivatives of Cyclodextrins

Cationic CD derivatives containing amino and alkylamino groups seem to be the most promising chiral selectors in CE. A wide variety of mono- and poly-substituted amino derivatives of CDs are described in the review by Croft and Bartsch.[13] Since publication of this review more than 40 papers and patent applications have appeared concerning this type of CD derivative. Synthetic methods have been developed which allow the introduction of amino substituents regioselectively onto the primary or secondary hydroxy side and also the preparation of designed AB, AC, AD, ABC *etc.* derivatives by using appropriate capping reagents. All these derivatives are of great interest for a study of the chiral recognition mechanism of CD hosts.

The general scheme for synthesis of amino-CDs involves the selective activation of a specific hydroxy group *via* tosylation and further substitution of the tosyl moiety directly with amines or *via*

an azide intermediate which is further reduced to the corresponding amine by conventional methods

A number of other positively charged nitrogen-, phosphorus- and sulfur-containing CD derivatives have been described [14] The quaternary ammonium, sulfonium and phosphonium salts are interesting chiral selectors, in that they possess a pH-independent positive net charge, which can be easily modified by simple variation of the degree of substitution

A number of interesting examples of chiral recognition by cationic CD derivatives have been published In the early 80s Breslow and Czarnik reported that β-CD modified with a pyridoxamine moiety on its secondary hydroxy side produced amino acids with opposite chirality to that produced from the corresponding 6-*O*-modified β-CD in transamination reactions [15] Murakami and co workers synthesized 6- and 3-acetylamino-3-deoxy-β-CDs and studied their comparative chiral recognition abilities towards racemic organic acids They established that the chiral recognition of 3-acetylamino-3-deoxy-β-CD was markedly higher than that of both the parent β-CD and its 6-acetylamino-6-deoxy-derivative towards (±)-mandelic acid, (±)-methyl mandelate and (±)-*N*-acetyl-α-phenylglycine [16] Recently, Brown *et al* reported that the aminopropylamino β-CD discriminates between the enantiomers of sodium 2-phenylpropanoate much better than unmodified β-CD Furthermore, the chiral recognition of this selector is enhanced dramatically by its complexation with Ni^{2+} [17] These and a number of other interesting examples of the chiral recognition of positively charged CD derivatives will stimulate research in the near future on the use of these materials as chiral selectors in CE At present, only 'Quaternary β-Cyclodextrin' is commercially available (from Supelco)

3.2 Anionic Derivatives of Cyclodextrins

More than fifteen anionic CD derivatives are commercially available, unlike the foregoing cationic derivatives This fact perhaps explains the extensive applications of these derivatives as chiral selectors in CE

It should be mentioned that all commercially available anionic CD derivatives are a mixture of components with a different degree of substitution Manufacturers characterize these products using an average degree of substitution (D S) estimated on the basis of elemental analysis Furthermore, most of these derivatives contain substantial amounts of native CDs There are only a few exceptions where definitely substituted anionic CDs have been synthesized [13] More detailed information about the composition and electrophoretic mobilities of individual components of charged CD mixtures is of vital interest for standardization (batch-to-batch, source-to-source *etc*), optimization and mechanistic studies of CE enantioseparations using charged CDs

CE in both direct and indirect detection mode can provide useful information about the composition and mobilities of individual components of a complex mixture of charged CDs CE profiles of three commercially available charged CD derivatives (a) carboxymethyl-β-CD (CM-β-CD), (b) sulfobutyl ether of β-CD (SBE-β-CD) and (c) sulfoethyl ether of β-CD (SEE-β-CD) are given in Fig 2 [18] The CE profiles of SEE-β-CD and SBE-β-CD are in agreeement with the ion-spray and MALDI-TOF mass spectra of these mixtures The latter techniques allow the CE peaks to be identified and provide information about the tentative quantitative content of each component in the mixture (Fig 3) [19]

4 Cationic Cyclodextrin Derivatives as Chiral Selectors in CE

As already mentioned, a number of well-defined positively charged CD derivatives have been described and interesting chiral recognition of these derivatives is also known [15–17] Nevertheless, there are only two published papers concerning the use of cationic CDs as chiral selectors in CE [8 20]

The paper by Terabe[8] is the first report about the use of charged CDs as chiral selectors in CE A number of dansylated racemic amino acids were resolved using mono-(6-β-aminoethylamino-6-deoxy)-β-CD in this study

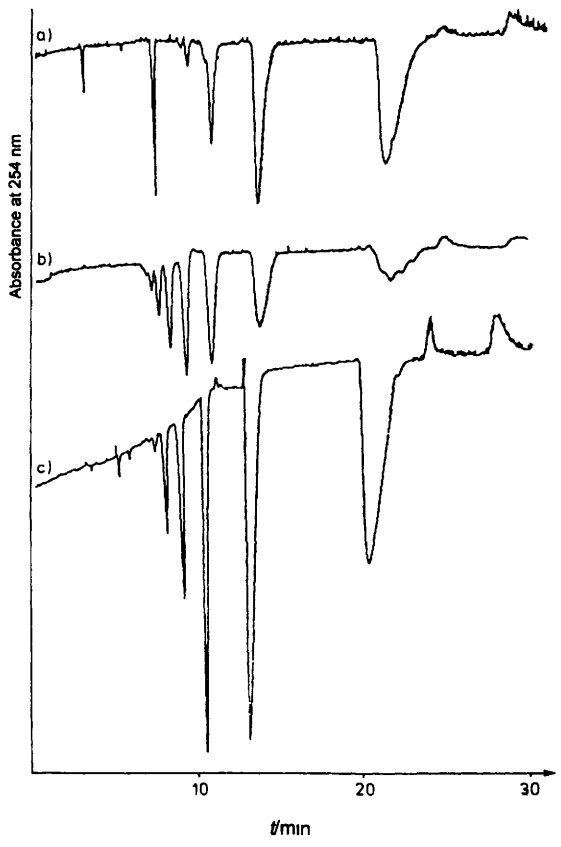


Figure 2 Electropherograms of (a) CM β CD, (b) SBE β CD and (c) SEE β CD used as chiral selectors Polyacrylamide coated fused silica capillary with 41 cm effective length was filled with 30 mmol l $^{-1}$ benzoic acid buffer at pH 6 0 5 mmol l $^{-1}$ solution of the CD derivatives were injected hydrostatically on the cathodic end and detected using the indirect detection technique at 254 nm The field strength was maintained at −345 V cm^{-1} (Reproduced by permission from *J Chromatogr A*, 1995, **704**, 234)

A more detailed study of cationic CD derivatives, particularly 6-methylamino-β-CD and 6A-,6D-dimethylamino β-CD, as chiral selectors for the enantioseparation of racemic 2-hydroxy acids was reported by Fanali *et al* [20] Several neutral cyclodextrins such as native β-CD, heptakis-(2,6-di-*O*-methyl)-β-CD and 2-hydroxypropyl-β-CD were also studied as references Higher chiral recognitions of both aminoalkyl derivatives were obvious for all the compounds studied For example, 6*A*-,6*D*-dimethylamino-β-CD resolved almost completely racemic *meta*-methylmandelic acid and 3,4-dimethylmandelic acid at a concentration as low as 1 mmol l $^{-1}$, while none of the neutral CDs exhibited a chiral recognition for any of these compounds even at a concentration of 80 mmol l $^{-1}$ in the buffer used This improvement of the chiral recognition was ascribed to the stabilization of inclusion complexes and enhanced enantioselectivity of binding due to additional electrostatic interactions between amino (chiral selector) and carboxy (analyte) groups Another, possibly even more important factor, is the self-mobility of the positively charged chiral selector this probably moves in an opposite direction to the analyte in this case

Several positively charged CD derivatives have been used in our laboratory for enantioseparation of racemic mandelic acid and its derivatives as well as of racemic 1,1′-binaphthyl-2,2-diyl hydrogen phosphate and 1,1-binaphthyl-2,2-diamine [21 22] The quaternary methyl- and ethyl-ammonium salts of β-CD exhibited chiral recognition in these studies but it is difficult to attribute this effect to the additional electrostatic interactions, because the former compound is resolvable also using neutral derivatives of β-CD with enhanced water solubility[20] and the latter using native β-CD[21 22] also As for mono-6-amino-6-deoxy-β-CD, it seems to possess better chiral recognition ability towards mandelic acid than the native β-CD, which did not show any sign of enantioseparation even at the solubility limit of 20 mmol l $^{-1}$ Mono-6-ethylamino-β-CD exhibits lower chiral recognition in comparison to its primary analogue

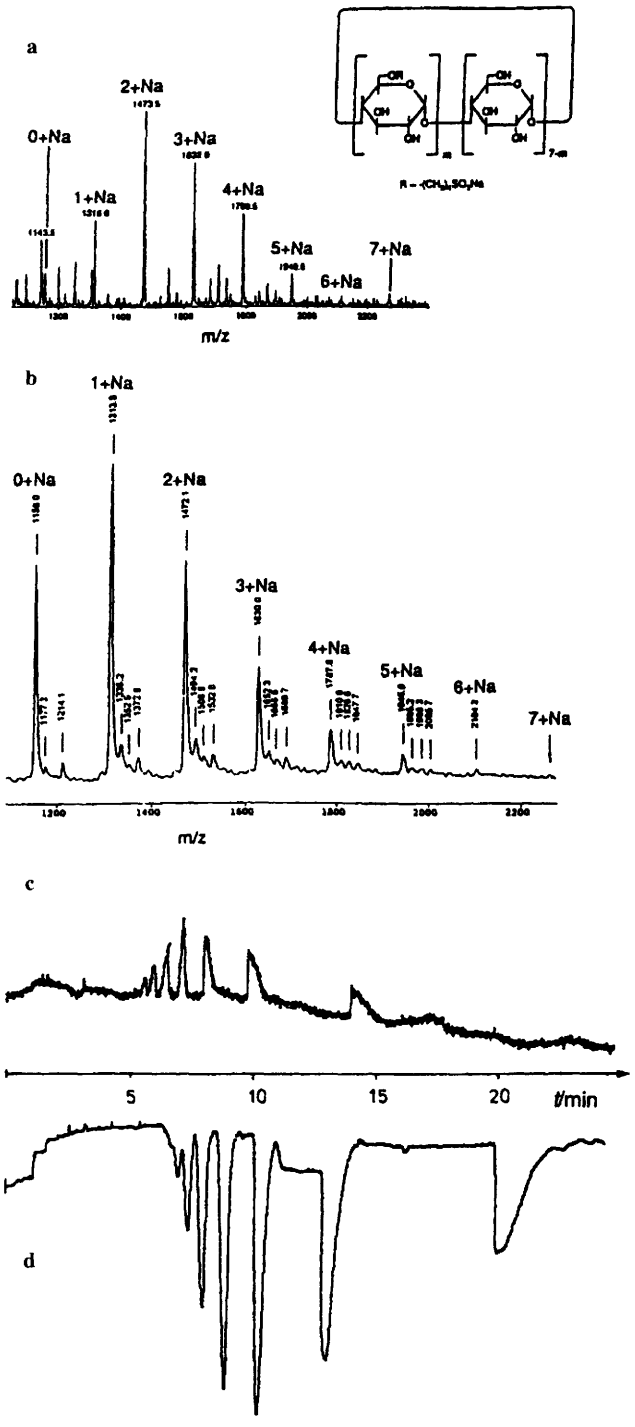


Figure 3 Comparison of **a** ion-spray and **b** MALDI-TOF mass spectra of SBE-β-CD and CE: **c** direct (190 nm in 50 mmol l^{-1} phosphate buffer, pH 4.6) and **d** indirect detection (254 nm in 30 mmol l^{-1} benzoic acid buffer, pH 4.6) of SBE-β-CD.

In summary, despite the well established synthetic methodologies and the number of examples of fascinating chiral recognition of cationic CD derivatives, their potential as chiral selectors in CE has not yet been completely explored.

5 Anionic Cyclodextrin Derivatives as Chiral Selectors

An anionic CD derivative, mono-2-*O*-carboxymethyl-β-CD, was used as the moving pseudostationary phase in CE ten years ago by Terabe *et al.*[9] No chiral recognition was reported at that time. In 1993 Smith described the enantioseparation of some chiral analytes using carboxymethyl-β-CD (CM-β-CD) as chiral selector at pH 12.4.[23] A more detailed study of carboxylated CDs as chiral selectors in CE was performed by Engelhardt and Schmitt.[24,25] At the same time CD sulfates and alkyl sulfates were used by our group and by others as chiral selectors for enantioseparation.[18,21,22,26–35] The following subsections point out more clearly the advantages of charged CDs over neutral chiral selectors.

5.1 Enantioseparation of Basic Racemates

The enantioseparation of a mixture of basic drugs containing doxylamine, ephedrine, dimethindine and propanolol using CM-β-CD as a chiral selector was reported in ref. 24. The hydrogen bonding capacity of the polar carboxymethyl function was considered to be the dominant contributor in the higher resolution of enantiomers by this chiral selector in comparison to uncharged CDs. The separation of dimethindene enantiomers was further optimized by using a shorter capillary (7 cm effective length) and an analysis time of less than 1 minute was achieved. To suppress the undesirable effect of the electroosmotic flow (EOF) on the separation efficiency polyacrylamide coated capillaries were used later for enantioseparation by the same group. The use of CM-β-CD as a carrier for positively charged analytes towards the anode has been demonstrated and ion pairing is considered to be the intrinsic mechanism of selector–solute interactions.[24]

Our studies show that ionic CD derivatives, especially CD alkyl sulfates, exhibit a chiral recognition ability at extremely low concentration in the run buffer.[18,21,22,26–30] The enantiomeric resolution of a mixture of racemic dimethindene, mianserine and mefloquine is shown in Fig. 4 as an example. The high countercurrent mobility of the chiral selector seems to be the most important factor responsible for the high effectiveness as mentioned in ref. 26. Basic racemates resolved using β-CD sulfates and alkyl sulfates as chiral additives are summarized in Table 1.

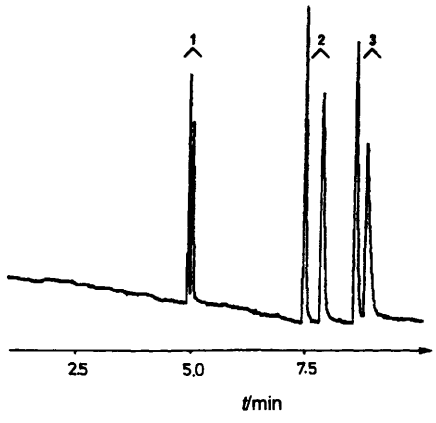


Figure 4 Chiral separation of (1) dimethindene, (2) mianserine and (3) mefloquine. Conditions: 50 mmol l^{-1} phosphate buffer, pH 3.10; +400 V cm^{-1}; 43 μA; detection at 210 nm, SBE-β-CD concentration: 80 μmol l^{-1}.
(Reproduced by permission from *Electrophoresis*, 1994, **15**, 804).

The validation and optimization of the separation seems to be important in order to realize the maximum stereoselective recognition ability of charged chiral selectors. An illustration in this respect is the resolution of the ephedrine enantiomers using SBE-β-CD as the chiral selector. Baseline separation of enantiomers was achieved with 1.5 mmol l^{-1} SBE-β-CD at pH 2.4,[33] whereas 40 mmol l^{-1} SBE-β-CD was required at pH 10.0.[32]

Damage of the peak form, exceedingly long elution or no elution at all of basic compounds was reported using SBE-β-CD as a chiral selector. The addition of organic modifier in the run buffer[27] can be helpful in this case. On the other hand no substantial interaction of negatively charged chiral selectors with the bare silica capillary wall is to be expected since the latter is also negatively charged in the pH range 2.5 – 10.0.

5.2 Enantioseparation of Neutral Racemates

One important merit of charged CDs as chiral selectors in CE is the enantioseparation of neutral chiral drugs. Smith reported the

Table 1 Enantioseparation of selected basic racemates using SBE-β-CD

Compound	SBE-β-CD (mmol l^{-1})	Buffer	α_{rel}	Reference
Clenbuterol	1 00	50 mmol l^{-1} Phosphate, pH 3 0	1 01	26
Dimethindene	0 08	"	1 02	"
Etilefrin	1 00	"	1 02	"
Imafen	1 00	"	1 05	"
Isoprenaline	1 00	"	1 01	"
Lofexidine	1 00	"	1 03	"
Mefloquine	0 08	"	1 03	"
Metomidate	1 00	"	1 03	"
Mianserine	0 04	"	1 03	"
Bifonazole	0 10	50 mmol l^{-1} Phosphate – 20% MeOH, pH 3 0	1 01	27
Econazole	0 10	"	1 06	"
Enilconazole	0 10	"	1 04	"
Miconazole	0 10	"	1 05	"
Ornidazole	0 10	"	1 00	"
Ketoconazole	0 10	50 mmol l^{-1} Phosphate – 40% MeOH, pH 3 0	1 05	"
1,1-Binaphthyl-2,2 diamine	0 50	50 mmol l^{-1} Phosphate, pH 3 0	1 05	21
7 Methylmianserine	0 10	"	1 06	29
7 Chloromianserine	0 10	"	1 06	"
8-Methylmianserine	0 10	"	1 19	"
8 Methoxymianserine	0 10	"	1 16	"
8 Chloromianserine	0 10	"	1 21	"
8 Fluoromianserine	0 10	"	1 09	"
8-Hydroxymianserine	0 10	"	1 16	"
9-Methylmianserine	0 10	"	1 18	"
cis 10-Hydroxymianserine	0 50	"	1 02	"
trans 10 Hydroxymianserine	0 10	"	1 02	"
12 Chloromianserine	0 10	"	1 08	"
13-Methylmianserine	0 10	"	1 08	"
N-Desmethylmianserine	0 10	"	1 07	
Phenylalanine, dansylated	5 00	20 mmol l^{-1} Borate, pH 7 0		32
Ephedrine[a]	40 00	20 mmol l^{-1} Borate, pH 10 0		"
Norephedrine[a]	40 00	"		"
Methylephedrine[a]	40 00	"		"
Pseudoephedrine[a]	40 00	"		"
Methylpseudoephedrine[a]	40 00	"		"
Ephedrine	1 50	200 mmol l^{-1} Borate, pH 2 5		33
Pseudoephedrine	1 50	"		"
Adrenaline	2 50	"		"
Noradrenaline	2 50	"		"
Tyrosine	5 00	"		"
DOPA	5 00	"		"
Salbutamol	2 00	20 mmol l^{-1} Citric acid–phosphate, pH 2 5		34
Cimaterol	2 00	"		
Atenolol	2 00	"		"
Clenbuterol	2 00	"		"
Terbutaline	2 00	"		"
Methylamphetamine	4 60	"		"
Methylmethoxyamphetamine	2 00	20 mol l^{-1} Citric acid–phosphate–5% Propan-1-ol		"

[a] Probably resolved in anionic form

enantioseparation of the neutral chiral compound named as GR 57 888X and developed by Glaxo Group Research as a possible drug entity [23] The chiral selector in this case was CM-β-CD The high pH (12 4) of the separation buffer may be responsible in this instance for a relatively high concentration of the chiral additive (25 mmol l^{-1}) which was required for the chiral separation

Engelhardt and Schmitt described the enantioseparation of a neutral oxazolidinone derivative, 1,1′-binaphthyl alcohol and uncharged hexobarbital using CM-β-CD, carboxyethylated-β-CD and succinylated-β-CD as chiral additives [24] It is interesting to mention the authors' view of the role of the alkyl spacer between the carboxylic acid function and the CD rim Comparing the chiral recognition ability of carboxymethyl- and carboxyethyl-β-CD the authors found that peak resolution is higher for hexobarbital and 1,1′-binaphthyl alcohol for the carboxyethyl compound and ascribed this effect to the spacer length Actually, the spacer length is not the only difference between these two derivatives CM-β-CD used in this study has an average D S of 3 6, whereas the D S of carboxyethyl-β-CD is *ca* 6 0 Under the conditions reported in this study (pH 5 8) the number of carboxylic groups per CD molecule will substantially affect the self-mobility of the chiral selectors, as well as their binding abilities and stereoselectivities

In our laboratory the resolution of the enantiomers of neutral thalidomide was achieved using 2 mmol l^{-1} SBE-β-CD as the chiral selector in 50 mmol l^{-1} phosphate buffer at pH 4 35 (Fig 5) [26] Recently, enantioseparation in the same solute–selector pair was described at pH 2 5 The neutral compound thalidomide should have extremely low mobility (which coincides with the EOF) at this pH Acceptable migration time has been reported which decreases surprisingly with increasing SBE-β-CD concentration [34]

Our results on the enantioseparation of neutral racemic compounds using alkyl sulfated CD derivatives[28] are summarized in Table 2 together with recently published data of Stalcup and Wu [35] All separations are performed in uncoated silica capillaries and the EOF is used as a carrier for the neutral analytes The separation of the thalidomide molecule and its neutral metabolites and the enantioseparation of them in the same run was recently achieved in our laboratory using a carboxymethylated β-CD as chiral selector As these few examples show, enantioseparation of neutral racemates is one of the most attractive fields of application of charged CD derivatives in CE

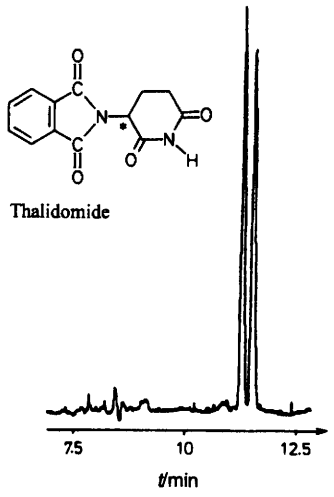


Figure 5 Chiral separation of racemic thalidomide. Conditions: 50 mmol l^{-1} phosphate buffer, pH 4.35; electric field strength +345 V cm^{-1}; detection at 210 nm, SBE-β-CD concentration: 2 mmol l^{-1}.
(Reproduced by permission from *Electrophoresis*, 1994, **15**, 804)

It should also be mentioned that CE with charged CD chiral selectors is not the only technique which permits the enantioseparation of neutral racemates. Micellar electrokinetic chromatography (MEKC) with charged chiral micelles, CD-modified MEKC with achiral micelles, biopeptides, charged chiral polymers and linear oligomers can also be used for the same purpose. Important advantages of charged CDs against the above-mentioned chiral additives seems to be the easy availability, simple control and management of ionic properties and mobility. The disadvantages of charged CDs as chiral selectors in CE include their availability only in one enantiomeric form, definite cavity size and relatively weak interactions with chiral compounds lacking an aromatic moiety. Some of these disadvantages may be overcome by designed derivatization of CDs.

5.3 Enantioseparations in Selector–Solute Pairs with the Same Net Charge

Taking into account a quite well established hypothesis that electrostatic interactions should substantially contribute to the chiral recognition of charged chiral selectors, selector–solute pairs possessing a net charge of the same sign cannot be optimal for achieving enantioseparation. Therefore, in the vast majority of studies concerning enantioseparations in CE, oppositely charged selector–solute pairs or charged chiral selector–neutral analyte pairs and *vice versa* have been used.

In our recent study[18] we showed that anionic β-CD derivatives, such as SEE-β-CD, SBE-β-CD, and CM-β-CD, beside the enantiomers of positively charged and neutral racemates, permit the resolution of the negatively charged analyte 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (Fig. 6).

Later the enantioseparation of other anionic racemates was achieved using anionic SBE-β-CD as chiral selector,[28] as well as enantioseparation in a pair: positively charged CD derivative–positively charged 1,1′-binaphthyl-2,2′-diamine.[21] ^{1}H NMR studies were

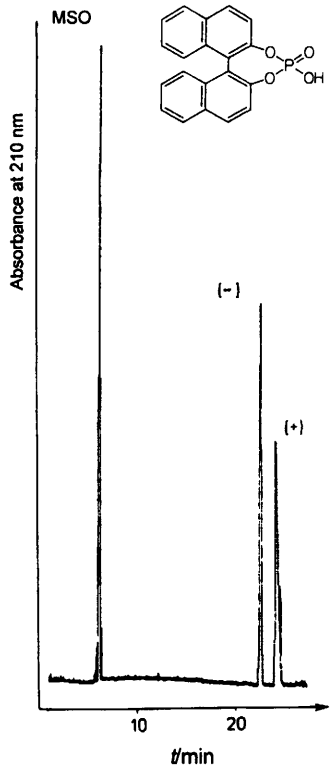


Figure 6 Electropherogram of 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate. Uncoated fused silica capillary with 43 cm effective length was filled with with 0.5 mmol l^{-1} SBE-β-CD – 50 mmol l^{-1} phosphate buffer at pH 6.0. The field strength was maintained at +400 V cm^{-1}. The sample was injected hydrostatically at the anode. The EOF was measured as the migration time of mesityl oxide (MSO).
(Reproduced by permission from *J. Chromatogr. A*, 1995, **704**, 234).

Table 2 Enantioseparation of various uncharged compounds using sulfated β-CD as chiral selectors

Solute	pH	SO_3-β-CD (D.S.≈7.0) [SO_3-β-CD] (%)	R_s	SBE-β-CD (D.S.≈4.0) [SBE-β-CD]/mmol l^{-1}	α	References
Benzoin	6.0			2	1.02	28
Benzoin methyl ether	6.0			2	1.06	"
Enilconazole[a]	9.0			1	1.02	"
5-Methyl-5-phenylhydantoin[b]	4.5			2	1.02	"
Metomidate[a]	9.0			1	1.02	"
Thalidomide	4.5			2	1.03	26
Hexobarbital[b]	6.0	—	—	2	1.06	28
1-Methyl-5-ethyl-5-propylbarbital[b]	6.0	—	—	5	1.04	"
Mianserine[a]	9.0			0.5	1.04	"
9-Methyl-$\Delta^{5(10)}$-1,6-dione	6.0	2	2.94			35
Phensuximide	7.0	3	1.94			"
5-(4-Hydroxyphenyl)-5-phenylhydantoin[b]	8.0	2	2.94			"
5-(4-Methylphenyl)-5-phenylhydantoin[b]	8.0	2	2.96			"
5-Cyclobutyl-5-phenylhydantoin[b]	8.0	2	3.95			"
Indapamide	7.0	4	1.50			"
1,1′-Binaphthol	8.0	2	2.72			"
Benzoin	7.0	2	1.77			"
Hydroxybenzoin	8.0	2	4.40			"
Troger's Base[a]	8.0	2	2.44			"

[a] Originally basic compound but neutral at this pH. [b] Originally weak acid but neutral at this pH.

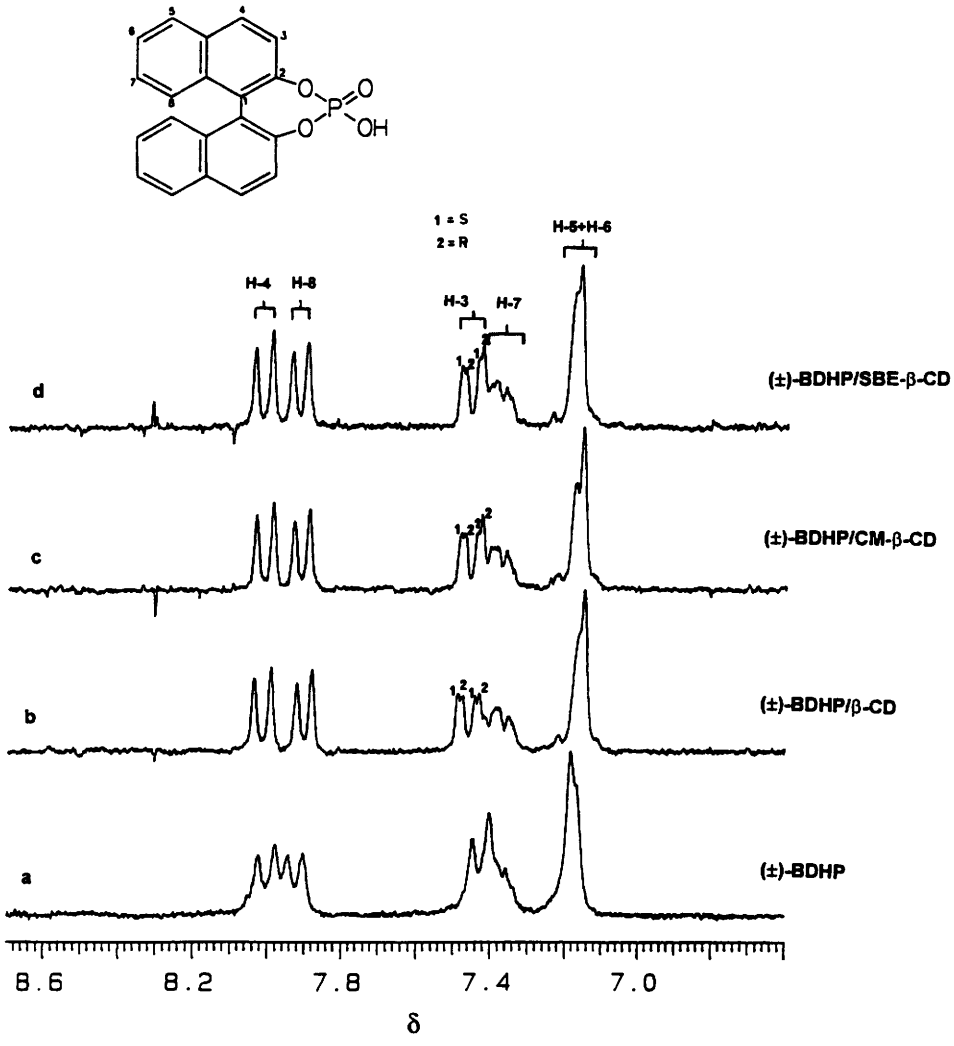


Figure 7 ^{1}H NMR spectra of (a) (±)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate and of its equimolar solutions with (b) β-CD, (c) CM-β-CD and (d) SBE-β-CD.

performed to get more detailed information about the selector–solute interactions in these quite surprising cases. The ^{1}H NMR spectra of (a) (±)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (**1**) and its equimolar mixtures with (b) β-CD, (c) CM-β-CD and (d) SBE-β-CD in D_2O+10% CD_3OD at pD 6.0 are depicted in Fig. 7. As these spectra show the 3-H doublet in the ^{1}H NMR spectra of (±)-**1** is split owing to the nonequivalence of the complexation-induced chemical shifts for the (*R*)-(−) and (*S*)-(+) enantiomers of (±)-**1** in the presence of all CDs. This means that the chirality of compound **1** in the anionic form in this case is recognized with neutral β-CD, as well as with anionic CM- and SBE-β-CD derivatives.

5.4 Reversal of the Enantiomer Elution Order

One of the important disadvantages of CD-type chiral selectors is the availability of these natural macrocycles only in one enantiomeric form. This means that no simple reversal of the enantiomer elution order is possible by using the chiral selector of the opposite configuration. This would be desirable in order to detect a minor enantiomeric component in front of the major one in non-racemic mixtures of enantiomers. Engelhardt and Schmitt[24,25] developed several techniques for the reversal of the enantiomer elution order in CE depending on the type of capillary used (coated and uncoated), direction of the EOF, type and concentration of the chiral selector and pH of the separation buffer in combination with reversed polarity of the electrodes.

Three different types of reversal of enantiomer elution order were observed in our laboratory using charged and uncharged CDs as chiral selectors in uncoated silica capillaries.[22] As Fig. 7 shows,

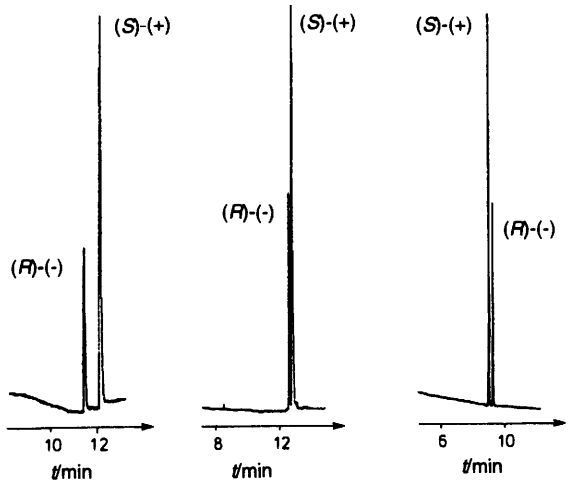


Figure 8 Enantioseparation of (±)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate with (a) β-CD (b) CM-β-CD and (c) SBE-β-CD at pH 3.7, electric field strength 400 V cm^{-1}. Detection at the anodic end of the capillary.

native β-CD, CM-β-CD and SBE-β-CD are not different from each other qualitatively in chiral recognition of (±)-**1**. In particular, the magnitude of the complexation-induced chemical shift of 3-H is higher for (*S*)-(+)-**1** than for (*R*)-(−)-**1** using all these CDs. In contrast to this result, the migration order for the enantiomers of **1** is similar for β-CD and CM-β-CD [(*R*)-(−) before (*S*)(+)] but reversed for SBE-β-CD (Fig. 8). The higher mobility of the multiply

negatively charged chiral selector compared to the mobility of the anionic chiral substance in the same direction may be the reason for the observed reversal of enantiomer elution order.

To confirm the higher self-mobility of SBE-β-CD in comparison to **1** at pH 3.7 the following experiments were performed. (1) The separation capillary was filled with a 2.5 mmol l^{-1} solution of SBE-β-CD and the selector-free buffer was added to the cathodic vial. No enantioseparation of **1** was observed in this case [Fig. 9(a)]. (2) Both the separation capillary and the cathodic vial were filled with

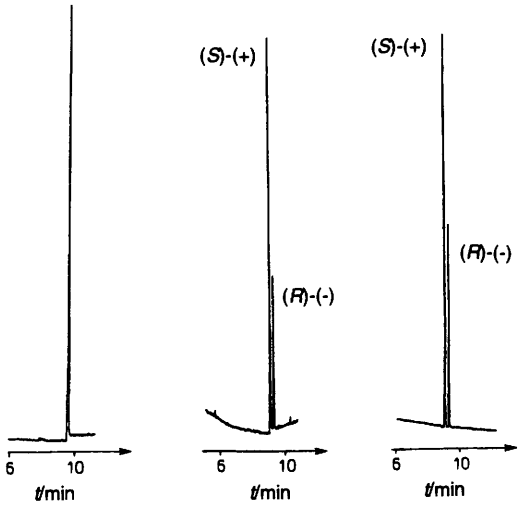


Figure 9 Enantioseparation of 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate with (a) SBE-β-CD (only in the capillary), (b) SBE-β-CD (in the capillary and cathodic vial) and (c) SBE-β-CD (only in the cathodic vial) at pH 3.7. Electric field strength 400 V cm^{-1}. Detection at the anodic end of the capillary.

a 2.5 mmol l^{-1} solution of SBE-β-CD in the buffer solution. The enantioseparation of **1** was observed in this experiment with the elution order (*S*)-(+)-**1** before (*R*)-(−)-**1** [Fig. 9(b)]. (3) The separation capillary was filled with chiral selector-free buffer and a 2.5 mmol l^{-1} solution of SBE-β-CD in the buffer was added only to the cathodic vial. The enantioseparation observed in this chiral selector free capillary [Fig. 9(c)] is the same as in case (2). Thus, the solute complexed by SBE-β-CD migrates faster; hence the mobility of the preferentially bonded enantiomer is higher than the mobility of the less bonded one. The opposite is true for the native β-CD and CM-β-CD. Therefore, the enantiomer elution order in the case of SBE-β-CD is the reverse of that of β-CD and CM-β-CD.

The study of the pH dependence of the mobilities of CM-β-CD[28] and **1** leads to another type of reversal of enantiomer elution order. As mentioned above, at pH 3.0 the electrophoretic mobility of CM-β-CD ($\mu_{selector}$) is lower than the mobility of **1** (μ_{solute}). At pH 5.0 this relation reverses for the same pair ($\mu_{selector} > \mu_{solute}$). Thus a reversal of the enantiomer elution order should be observed between these two pH values. Indeed, as Fig. 10, shows the peak coalescence of **1** occurs at pH 4.3 where the effective mobilities of the chiral solute and selector are equal ($\mu_{selector} \approx \mu_{solute}$) and reversal of the elution order appears above pH 4.3 ($\mu_{selector} > \mu_{solute}$). Neither the reversal of the polarity of the high voltage supply nor the elimination of the EOF is required to observed this effect.

The third type of the reversal of the enantiomer elution order is in principle similar to that described in refs. 24 and 25. The last technique has been considered to be useful only with charged chiral selectors and requires the elimination of the EOF by coating the inner wall of the capillary. In the present extension of this technique, instead of elimination of the EOF, it is used as counter-current flow to the electrophoretic mobility of the chiral solute and the reversal of the enantiomer elution order is observed not only using charged but also with neutral CDs (Fig. 11). Thus, the number of chiral selectors and racemic solutes useful for this technique is expanded substantially. This seems to be the most universal and predictable technique for the reversal of the enantiomer elution order applicable for a wide variety of chiral solutes and chiral selectors.

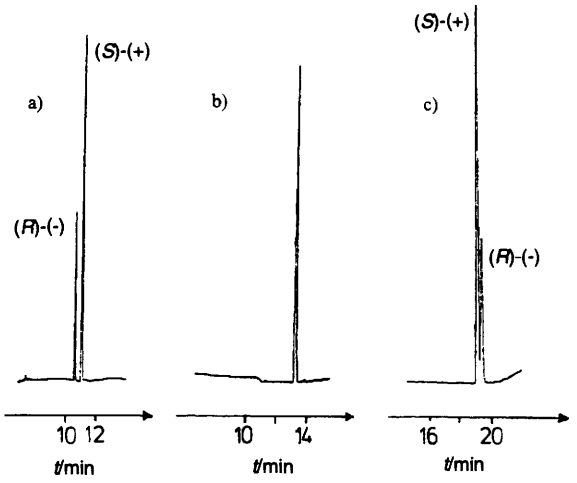


Figure 10 The pH-dependent reversal of the enantiomer elution order of 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate using 5.0 mg ml^{-1} CM-β-CD (in the capillary and cathodic vial): (a) pH 3.0, (b) pH 4.3 and (c) pH 5.0. Electric field strength 400 V cm^{-1}. Detection at the anodic end of the capillary.

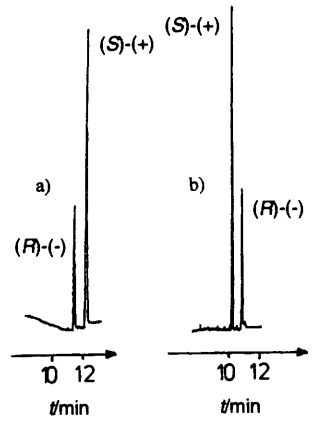


Figure 11 The reversal of the enantiomer elution order of 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate in uncoated fused silica capillary using 2.5 mg ml^{-1} β-CD: (a) electric field strength −400 V cm^{-1} at pH 3.3 and (b) (anodic vial was filled with the chiral selector in addition to the separation capillary) electric field strength +400 V cm^{-1} at pH 6.5.

The techniques of the reversal of the enantiomer migration order summarized in this section make the disadvantage of CD chiral selectors less critical.

5.5 Enantioseparation in Counter-current Modes

One of the important advantages of CD-type chiral selectors in CE is their UV transparency. From HPLC studies it is known that the introduction of a phenyl- or a naphthyl-moiety substantially improves the chiral recognition abilities of CDs. These derivatives have some potential also in CE but their strong UV absorbance makes them incompatible with the most commonly used UV detectors.

The self migration ability of charged CD derivatives in a definite direction enables separation schemes to be realized in which the chiral selector does not appear simultaneously with the chiral solute in the detector cell. One such technique was described in ref. 26. The separation of the chiral dimethindene enantiomers was achieved in this study (Fig. 12) in the counter-current flows of discrete zones of a 5 mmol l^{-1} SBE-β-CD solution and racemic dimethindene solution. After applying the electric field the zone of the chiral selector moves from the cathode to the anode, passes the detector cell and comes in contact with the chiral solute before it reaches the detector cell. The resolved enantiomers are detected when they pass the selector-free detector cell. Another scheme

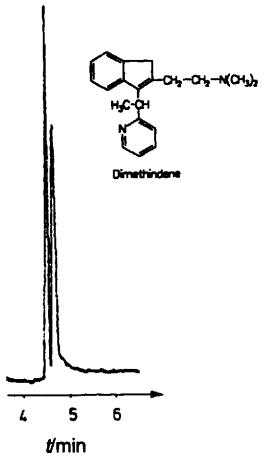


Figure 12 Separation of dimethindene enantiomers in counter-current mode. Conditions: 50 mmol l^{-1} phosphate buffer, pH 3.0; +420 V cm^{-1}; SBE-β-CD concentration: 5 mmol l^{-1}.
(Reproduced by permission from *Electrophoresis*, 1994, **15**, 804).

which permits the same kind of analysis to be performed could be the use of branched capillaries. The chiral selector will enter the separation capillary in front of the detector cell and migrate in the anodic direction. If the EOF (in the case of uncoated capillaries) and the distance between the coupling point of capillaries and the detector cell are appropriately chosen, this technique should prevent the appearance of the chiral selector in the detector cell.

6 NMR Studies of the Chiral Recognition Mechanism of Charged CDs

NMR spectroscopy is recognized to be the most useful instrumental technique for the study of CD complexes with various guests:

(1) This technique provides considerable information about the environment of individual atoms and intermolecular interactions and thus provides data about the structure and molecular dynamics of complexes.
(2) NMR spectroscopy allows a clear differentiation between inclusion and any other possible external interactions. This is an important advantage of this technique over all others, which are more global and do not provide any convincing proof for the inclusion.
(3) Enantiotopic signals should be distinguishable in a chiral environment in NMR spectra. This allows racemic samples to be used for the study of the enantioselective binding parameters (binding constants, stoichiometry, free energy, enthalpy and entropy).

NMR spectroscopy can obviously provide useful information about complexation patterns in the liquid phase; hence this technique should be used carefully.

The NMR signals observed in binary selector–solute solutions are the time-averaged signals of both the complexed and uncomplexed substances. This can give rise to signal nonequivalence in two ways. First, a difference in association constants between the two enantiomers can cause one enantiomer to be preferentially bound and subsequently more shifted. The chemical shift nonequivalence of this origin is related to the enantiomer recognition abilities in separation techniques like CE, HPLC, GC *etc*. Secondly, the two enantiomers may have the same association constants and therefore be bound in equal proportions, but the two diastereoisomeric complexes thus formed may have intrinsically different NMR spectra. The chemical shift nonequivalence with this origin causes signal splitting in NMR spectrum but does not correlate with abovementioned separation techniques. The calculation of the apparent binding constants (K_a) and the complexation-induced chemical shifts at saturation ($\Delta\sigma_c$) for individual enantiomers enables the differentiation between these two principally different cases.

To illustrate clearly how misleading NMR data can be, the details of the complexation of **1** with various CDs are described as an example. As Fig. 7 (Section 5.3) shows, native β-CD and its anionic derivatives CM-β-CD and SBE-β-CD form inclusion complexes with **1** with a measurable nonequivalence of the complexation-induced chemical shifts (CICS) between the enantiomers. CICS is sometimes used as a measure of the chiral recognition ability which seems to be incorrect in this particular case. The use of optically pure enantiomers of **1** in this study shows that the CICS is higher for one or the other enantiomer depending on which proton signal is measured. In particular, the comparison of CICS of β-CD complexes (the same is true for CM-β-CD) with (*S*)-(+)-**1** and (*R*)-(−)-**1** in solution shows that for the doublet at δ7.44 (3-H) the CICS is higher for (*S*)-(+)-**1**, whereas for the doublet at δ7.94 (8-H) the CICS is higher for (*R*)-(−)-**1**, as shown in Fig. 13. If the existence of only one type of complex is assumed in this solution then it can be inferred that the magnitude of the CICS does not always correlate with the complex stability. The results obtained using NMR spectroscopy become much more reliable when quantitative treatment of the spectral information is performed using Job's and Scott's techniques. The former provides information about the stoichiometry of the selector–solute complexes[21,29,30] and the latter about apparent binding constants (K_a) and CICS at saturation ($\Delta\delta_c$).[21,30] Averaged apparent binding constants (K_a) calculated using this technique for the complexes of (*S*)-(+)-**1** and (*R*)-(−)-**1** with β-CD and CM-β-CD are given in Table 3. These results are consistent with the enantiomer elution order of **1** in CE and allow some unusual effects observed in the latter technique to be explained.[21]

Another illustrative example of the utility of NMR spectroscopy for the study of the chiral recognition mechanism in CE is illustrated using the racemic imidazole derivative (±)-metomidate [(±)-MET]. As CE studies showed various native and derivatized CDs strongly differ from one each other in their chiral recognition behaviour towards this compound.[27] Among the native CDs at pH 3.5, α-CD does not exhibit any measurable chiral recognition ability even at a concentration as high as 50 mmol l^{-1} and γ-CD also hardly recognizes the chirality of (±)-MET, whereas native β-CD exhibits superior chiral recognition ability. These results are easily explained on the basis of the ^{1}H NMR spectra of (±)-MET and its equimolar complexes with α-, β- and γ-CDs given in Fig. 14. As this Figure shows α- and γ-CD do not cause any observable changes in the ^{1}H NMR spectrum of (±)-MET, whereas the effect is substantial in the case of β-CD. The downfield shift and a clear splitting of the CH quartet at δ6.26 and the upfield shift accompanied with a line splitting of the 2-H signals of the imidazole moiety at δ8.85 are the most remarkable in this case. As these data show, acceptable correlations can be observed between CE and NMR spectroscopy in some cases.

The following two examples demonstrate the effect of the ionic strength and the pH of the run buffer on the chiral recognition of CM-β-CD, observed in ^{1}H NMR and CE. The electropherograms of the CE enantioseparation of (±)-MET using 5 mmol l^{-1} CM-β-CD in buffer solutions of various ionic strength are depicted in Fig. 15 in parallel with the ^{1}H NMR spectra of equimolar solutions of the same selector–solute pairs in the same buffer solution. As this Figure shows, the migration time increases and the enantioseparation improves substantially on increasing the ionic strength of the buffer, whereas no definitive effects were observed in the ^{1}H NMR spectra. These results indicate that the intrinsic chiral recognition ability of CM-β-CD cannot be modified substantially and the improvement of the peak resolution is mainly caused by the change of the effective mobility of (±)-MET. In addition a better suppression of the electrophoretic dispersion with increasing ionic strength of the buffer can improve peak resolution.

NMR spectroscopy also provides important information on the pH-dependence of the selector–solute interactions. As in Fig. 15, the ^{1}H NMR spectra of equimolar solutions of (±)-MET–CM-β-CD at various pH values are depicted in Fig. 16 in parallel with the electropherograms of the enantioseparation obtained at the same pH in CE. A good correlation between CE and ^{1}H NMR spectroscopy is observed in this instance and no complexation-induced splitting of the spectral lines was observed at pH 2.4 in the ^{1}H NMR spectrum of the equimolar (±)-MET–CM-β-CD solution in D_2O and very poor enantioseparation was observed in CE. The signal of the

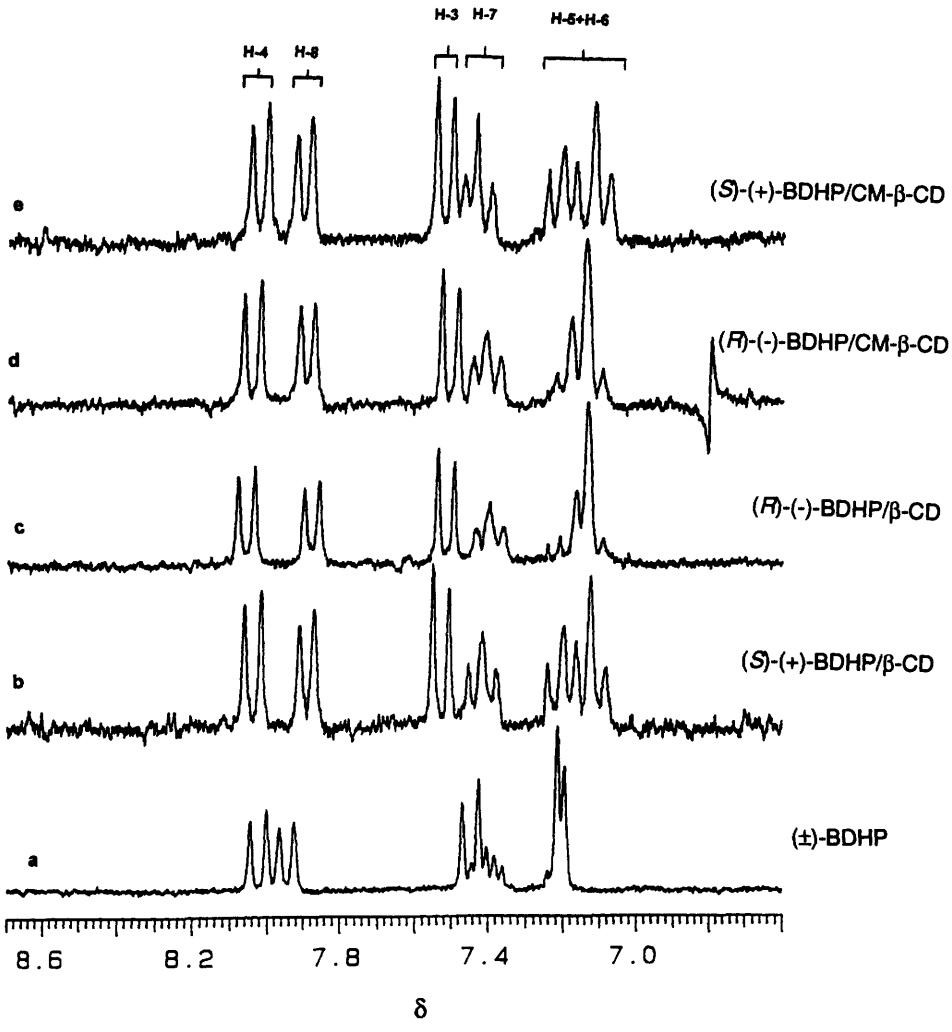


Figure 13 ^{1}H NMR spectra of (a) (±)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate, (b)(e) (*S*)-(+)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate, and (c), (d) (*R*)-(−)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate in the presence of a 3:1 molar excess of (b), (c) β-CD and (d), (e) CM-β-CD.

Table 3 Complexation-induced chemical shifts at saturation ($\Delta\nu_c$) and average apparent binding constants (K_a) for the complexes of (*S*)-(+)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate and (*R*)-(−)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate with β-CD and CM-β-CD.

CD	$\Delta\nu$ [(*R*)-(−)]/Hz		$\Delta\nu$ [(*S*)-(+)]/Hz		K_a [(*R*)-(−)] /mmol l^{-1}		K_a [(*S*)-(+)] /mmol l^{-1}		$\bar{K}_a$ [(*R*)-(−)] /mmol l^{-1}	$\bar{K}_a$ [(*S*)-(+)] /mmol l^{-1}	$\alpha_1=\frac{\bar{K}_a[(S)\text{-}(+)]}{\bar{K}_a[(R)\text{-}(-)]}$
	3-H	8-H	3-H	8-H	3-H	8-H	3-H	8-H			
β-CD	17.41	19.06	19.49	14.02	312	210	413	263	261	338	1.30
CM-β-CD	18.31	26.68	21.16	14.56	106	68	175	119	87	147	1.70

2-H proton of the imidazole moiety shifted markedly upfield and split ($\Delta\delta$ 0.02 ppm) in the same solution at pH 2.8, accompanied by substantial improvement of the peak resolution in CE. The upfield chemical shift of the 2-H signal of the imidazole moiety and the complexation-induced chemical shift nonequivalence between (±)-MET enantiomers as well as peak resolution in CE was enhanced by further increase of the pH up to 6.0.

As already mentioned above (Fig. 14) for (±)-MET–native CDs pairs a good correlation between the data from CE and ^{1}H NMR spectroscopy can be observed at pH 3.5. The similar correlation between these two techniques is observable when native β-CD is compared with anionic β-CD derivatives, such as CM-β-CD and SBE-β-CD (Fig. 17). As this Figure shows, in the ^{1}H NMR spectra all three CDs studied exhibit chiral recognition ability at pH 3.5 as well as at pH 6.0. All of them permit an effective enantioseparation of (±)-MET at pH 3.5 in CE, but only the anionic ones do so at pH 6.0. Quantitative calculations performed using Job's and Scott's techniques confirm that the chiral recognition ability of native β-CD at pH 6.0 is similar to that of SBE-β-CD and even better than that of CM-β-CD (Table 4).

To explain this discrepancy between results from CE and ^{1}H NMR spectroscopy, the enantioselectivity observed in CE (α) can be described by equation (3):

$$\alpha = 1 + k(K_2/K_1)(K_2 - K_1)(\mu_s - \mu_c) \quad (3)$$

where K_1 and K_2 are the apparent binding constants of the enantiomers (1) and (2) with a given chiral selector, μ_s is the mobility of the free solute, μ_c is the mobility of the selector–solute complex and k is a coefficient. In eqn. (3) term $(K_2/K_1)(K_2-K_1)$ characterizes the enantioselectivity and the strength of the selector–solute binding. The term $(\mu_s-\mu_c)$ reflects the effect of the mobility difference between free (μ_s) and bonded (μ_c) solute in enantioselectivity (α).

If we assume that the mobilities of the transient diastereoisomeric

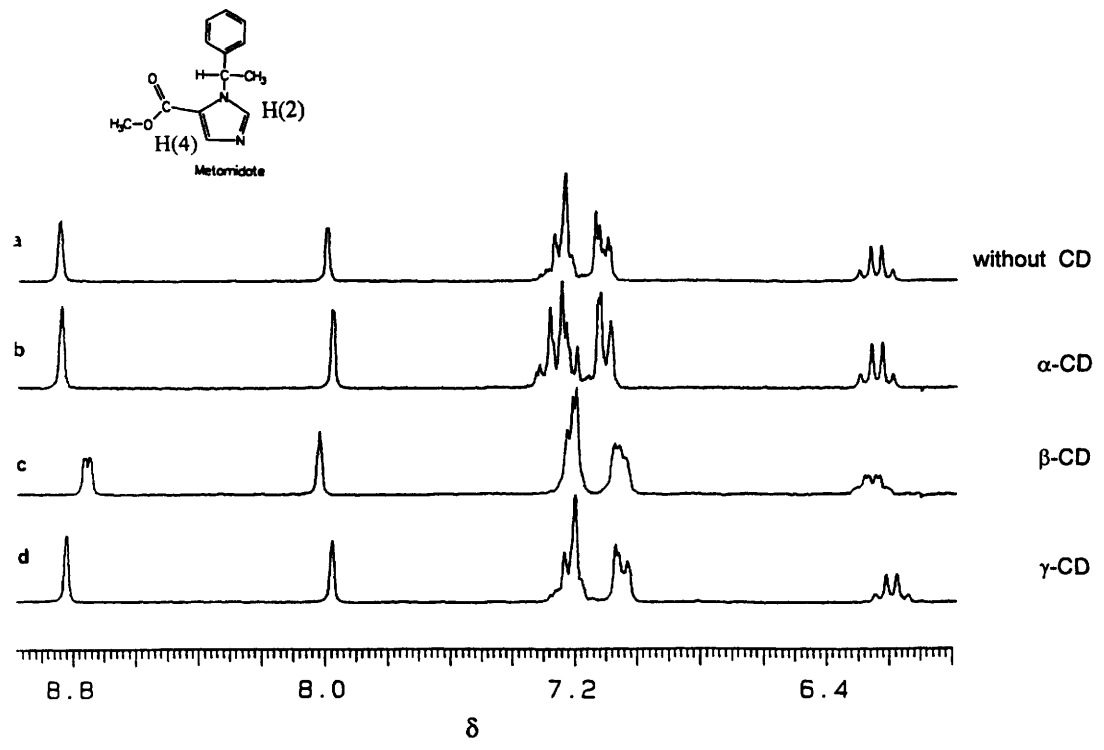


Figure 14 ^{1}H NMR spectra of equimolar mixtures (1 mmol l^{-1} each) of (±)-MET with α-, β- and γ-CD in deuterium oxide.

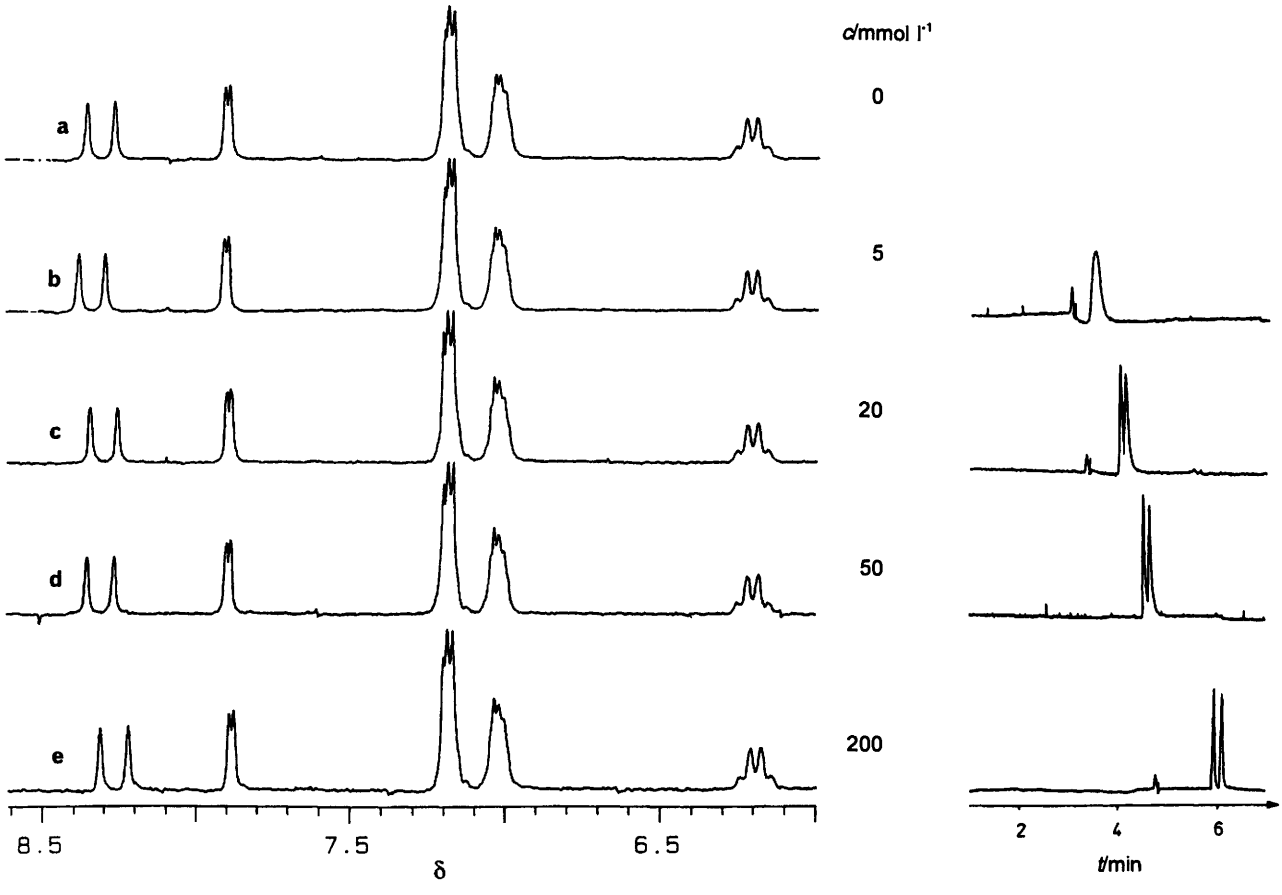


Figure 15 (Right) CE enantioseparation of (±)-MET at pH 6.0 using 5 mmol l^{-1} CM-β-CD in phosphate buffer of varying ionic strength. (Left) ^{1}H NMR spectra of equimolar solutions of (±) MET and CM-β-CD (1 mmol l^{-1} each) in phosphate buffer with varying ionic strength.

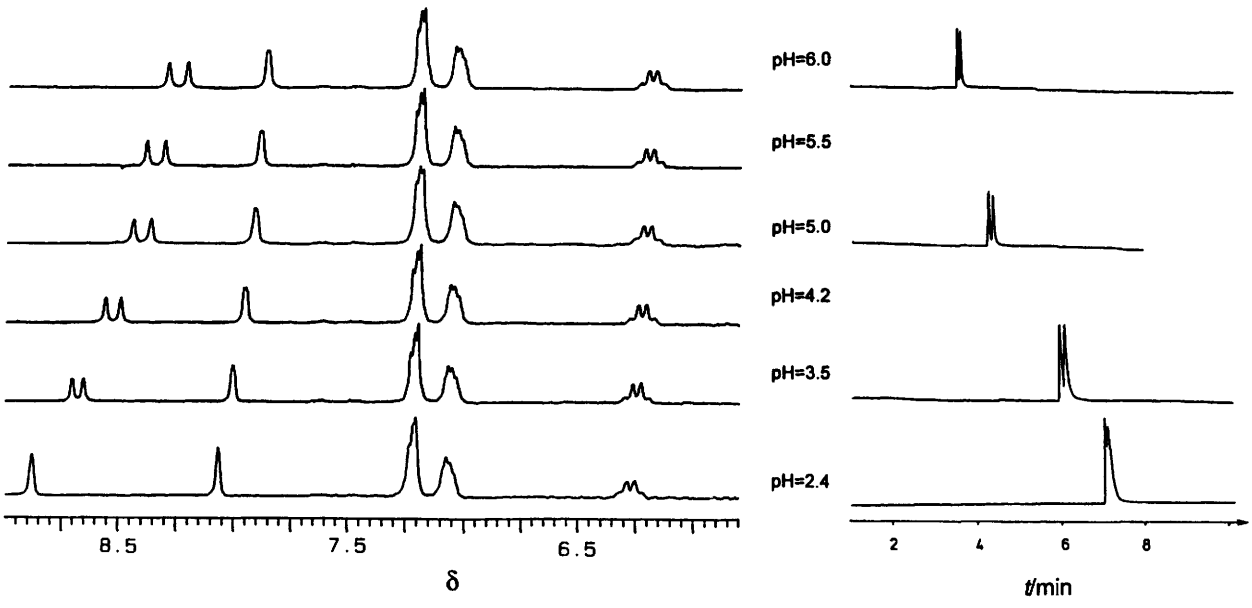


Figure 16 (Right) CE enantioseparation of (±)-MET using 5 mmol l^{-1} CM-β-CD in 50 mmol l^{-1} phosphate buffer at varying pH. (Left) ^{1}H NMR spectra of equimolar solutions of (±)-MET and CM-β-CD (1 mmol l^{-1} each) at varying pH.

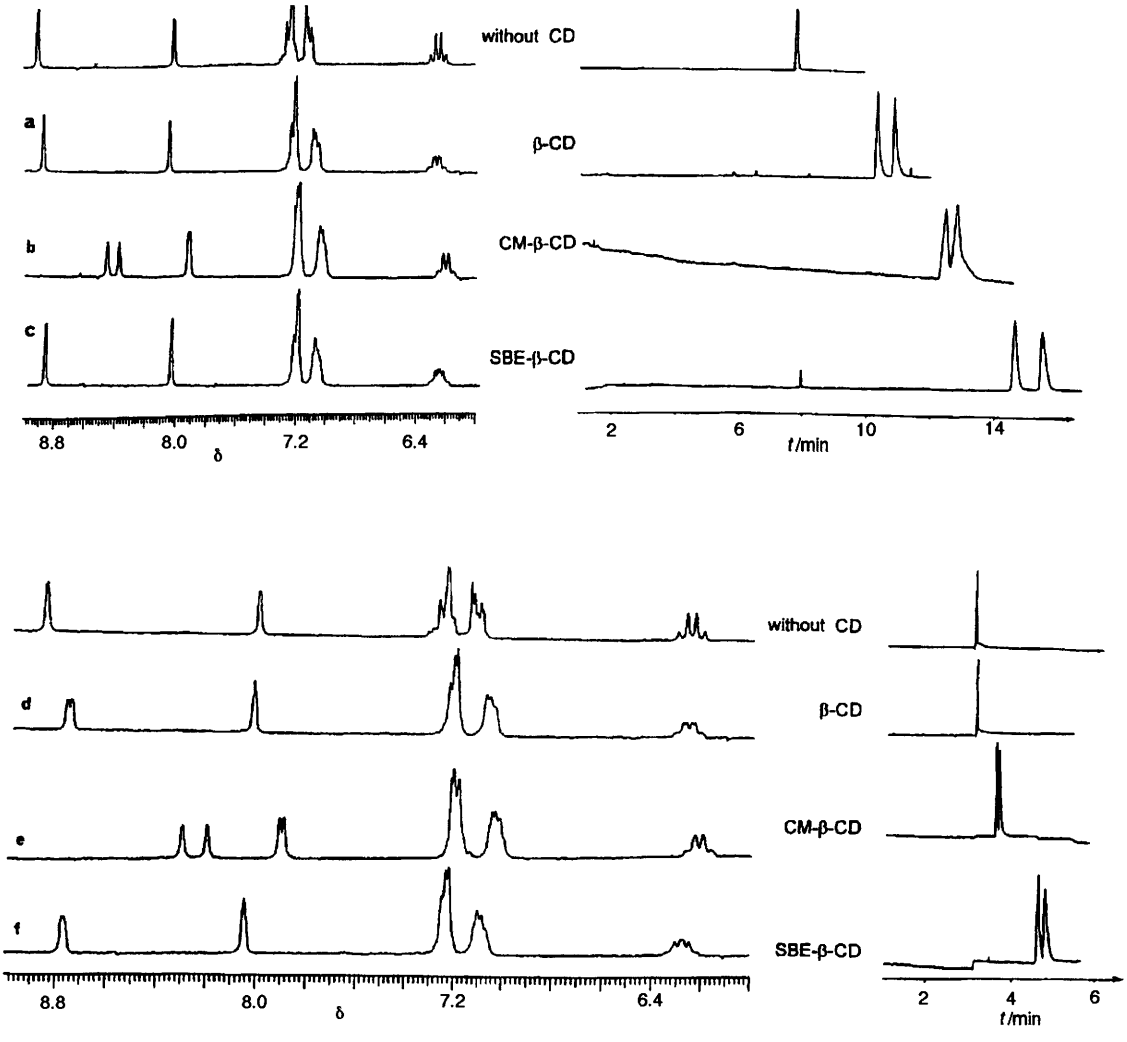


Figure 17 ^{1}H NMR spectra of equimolar solutions (10 mmol l^{-1} of each) of (±)-MET with (a), (d) β-CD, (b), (e) CM-β-CD and (c), (f) SBE-β-CD at (a–c) pH 3.5 and (d–f) pH 6.0 together with the CE enantioseparations with the same chiral selectors at the same pH.

Table 4 Complexation-induced chemical shift differences at saturation ($\Delta\nu_c$) and apparent binding constants (K_a) of (±)-MET with various CDs

CD	$\Delta\nu_c$/Hz		K_a/mmol l^{-1}		$\alpha_1=\frac{\bar{K}_{a2}}{\bar{K}_{a1}}$
	$\Delta\nu_{c1}$	$\Delta\nu_{c2}$	K_{a1}	K_{a2}	
β CD	20 9	16 5	483	655	1 36
CM β CD	210 8	218 1	350	423	1 21
SBE β CD	77 8	87 3	397	564	1 42

All binding parameters in case of CM β CD and SBE β CD were calculated on the basis of the 2 H signal at δ 8 85 and for β CD on the basis of the quartet at δ 6 14 due to methine protons in the CH–CH_3 bridge between the phenyl and imidazole moieties

complexes of the (*R*) and (*S*) enantiomers of a given chiral compound with a chiral selector do not differ from each other then from eqn (3) it is clear that enantioseparation is in principle possible when $K_1 \neq K_2$ and $\mu_s \neq \mu_c$ at the same time As Fig 17 shows, for noncharged CDs this condition is only possible at pH 3 5, whereas at pH *ca* 6 0 (±)-MET becomes almost neutral (p*K* 5–6) and it migrates in the electroosmotic flow (EOF) with almost the same effective mobility as noncharged CDs, *i e* $\mu_s=\mu_c$ and no enantioseparation is observed

In addition to the above mentioned methods, the nuclear Overhauser effect (NOE) has substantial potential for studying the intermolecular interactions and structure and dynamics of selector–solute complexes This technique can play an important part in the elucidation of chiral recognition mechanism using CD hosts

7 Conclusions

Capillary electrophoresis has developed explosively over the past few years as a powerful tool for enantiomeric analysis The fascination with a microanalytical technique which allows the use of nonhazardous chemicals to solve a problem which was recognized as most difficult in separation sciences a few years ago is high Many successful enantioseparations in CE have been achieved, whereas only a few studies have been performed to understand the basic mechanisms of chiral recognition using this technique

CE is actually not only a good complement but also a valuable alternative to HPLC (probably to GC and SFC too) in analytical scale enantioseparations Extremely high theoretical plate numbers, raggedness, versatility, cost effectiveness and short analysis time are milestones of this technique Charged CD derivatives offer a number of important advantages over neutral ones, which favour their rapidly increasing use as chiral selectors NMR spectroscopy is very valuable for optimization and method development in CE, for a better understanding of the chiral recognition mechanism and sometimes even for correction of results observed in CE Fundamental studies of the structure of selector–solute complexes will favour a designed synthesis of new types of chiral selectors CE should provide the impulse for a further increase in activity in supramolecular chemistry where it can serve as a state-of-the-art instrumental technique

8 References

1 G Pifferi and E Peruca, *Eur J Drug Met*, 1995, **20**, 15
2 S Hjerten, *Chromatogr Rev*, 1967, **9**, 122
3 R Virtanen, *Acta Polytechnica Scand*, 1974, **123**, 1
4 F E P Mikkers, F M Everaerts and Th P E M Verheggen, *J Chromatogr*, 1979, **169**, 11
5 J W Jorgenson and K D Lukacs, *Anal Chem*, 1981, **53**, 1298
6 E Gassmann, J E Kuo and R N Zare, *Science*, 1985, **230**, 813
7 S Fanali, *J Chromatogr*, 1989, **474**, 441
8 S Terabe, *Trends Anal Chem*, 1989, **8**, 129
9 S Terabe, H Ozaki, K Otsuka and T Ando, *J Chromatogr*, 1985, **332**, 211
10 A C Villiers, *R Acad Sci Paris*, 1891 **112**, 536
11 F Cramer, *Angew Chem*, 1952, **64**, 136
12 S Li and W C Purdy, *Chem Rev*, 1992, **92**, 1457
13 A P Croft and R A Bartsch, *Tetrahedron*, 1983, **39**, 1417
14 S M Parmeter, E E Allen and G A Hull, *US Pat* 3,453,257, *Chem Abstr* 1969, **71**, 91 821y
15 R Breslow and A W Czarnik, *J Am Chem Soc*, 1983, **105**, 1390
16 T Murakami, K Harata and S Morimoto, *Chem Lett*, 1988, 553
17 S E Brown, J H Coates, C J Easton, S J van Eyk, S F Lincoln, B L May, M A Stile, C B Whalland and M L Williams, *J Chem Soc Chem Commun*, 1994, 47
18 B Chankvetadze, G Endresz and G Blaschke, *J Chromatogr A*, 1995, **704** 234
19 B Chankvetadze, G Endresz and G Blaschke, M Juza, H Jakubetz and V Schurig, *Carbohydr Res*, in the press
20 A Nardi, A Eliseev, P Bocek and S Fanali, *J Chromatogr*, 1993, **638**, 247
21 B Chankvetadze, G Endresz, G Schulte, D Bergenthal and G Blaschke, *J Chromatogr A*, 1996, in the press Poster presentation at the 19th International Symposium on Column Liquid Chromatography, Innsbruck, Austria, May, 1995
22 B Chankvetadze, G Schulte and G Blaschke, *J Chromatogr A*, 1996, in the press
23 N W Smith, *J Chromatogr*, 1993, **652**, 259
24 T Schmitt and H Engelhardt, *Chromatographia*, 1993, **37**, 475
25 T Schmitt and H Engelhardt, *J Chromatogr A*, 1995, **697**, 561
26 B Chankvetadze, G Endresz and G Blaschke, *Electrophoresis* 1994, **15**, 804
27 B Chankvetadze, G Endresz and G Blaschke, *J Chromatogr A*, 1995, **700**, 43
28 B Chankvetadze, G Endresz and G Blaschke, *J Capillary Electrophoresis*, 1995, **2**, 235
29 B Chankvetadze, G Endresz, D Bergenthal and G Blaschke, *J Chromatogr A*, 1995, **717**, 245
30 G Endresz, B Chankvetadze, D Bergenthal and G Blaschke, *J Chromatogr A*, 1996, in the press
31 S Mayer and V Schurig, *Electrophoresis*, 1994, **15**, 835
32 C Dette, S Ebel and S Terabe, *Electrophoresis*, 1994, **15**, 799
33 R J Tait, D O Thompson V J Stella and J F Stobaugh, *Anal Chem*, 1994, **66**, 4013
34 A Aumatell, R Wells and D K Y Wong, *J Chromatogr A*, 1994, **686**, 293
35 W Wu and A M Stalcup, *J Liq Chromatogr*, 1995, **18**(7), 1289