

Review

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Recent progress in chiral separation principles in capillary electrophoresis

This review summarizes recent developments in the field of chiral separations by electromigration techniques including capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), isotachopheresis (ITP), electrokinetic chromatography (EKC), and capillary electrochromatography (CEC). This overview focuses on the development of new chiral selectors and the introduction of new techniques rather than applications of already established selectors and methods. The mechanisms of the different chiral separation principles are discussed.

Keywords: Enantiomer separation / Capillary electrophoresis / Review

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Abbreviations: **18C6H4**, 18-crown-6-tetracarboxylic acid; **AGP**, α_1 -acid glycoprotein; **AIBN**, 2,2'-azobis(isobutyronitrile); **BNHP**, (\pm)-1, 1'-binaphthyl-2,2'-diyl hydrogen phosphate; **CHARM**, charged resolving agent migration model; **CM- β -CD**, carboxymethyl- β -CD; **CSP**, chiral stationary phase; **DNP**, dinitrophenyl; **Dns**, dansylated; **EMO**, enantiomer migration order; **FA**, formamide; **FMOC**, 9-fluorenylmethoxycarbonyl; **HP- β -CD**, hydroxypropyl- β -CD; **NMF**, *N*-methylformamide; **NSAID**, nonsteroidal anti-inflammatory drug; **QA- β -CD**, quaternary ammonium- β -CD; **S- β -CD**, sulfated β -CD

1 Introduction

About 40% of the drugs in use are known to be chiral. It is well established that the pharmacological activity is mostly restricted to one of the enantiomers. In several cases unwanted side effects or even toxic effects can occur with the second enantiomer. Even if the side effects are not that drastic, the inactive enantiomer has to be metabolized and represents an unnecessary burden for the organism. The administration of the pure pharmacologically active enantiomers is therefore of great importance. The development of methods for enantiomer separation for controlling synthesis, for enantiomeric purity check, and for pharmacodynamic studies is attracting increasing interest. In addition to chromatographic methods, CE and more recently CEC are becoming increasingly attractive.

Several comprehensive review articles have appeared in recent years dealing with general aspects of chiral CE and applications of different chiral separation principles to

various compound classes [1–21]. This review will therefore focus on new techniques and new chiral selectors covering the literature from 1997 to date. The reader is referred to several specialized reviews on applications. Earlier articles are cited only if they explain mechanisms or contain the first mention of given separation principles. Chiral separation by CE can be performed either indirectly, using a chiral derivatization agent forming diastereomeric pairs, which can be resolved under achiral conditions, or directly, using chiral selectors as additives to the electrolyte. In CEC, a recent technique, similarly to HPLC, chiral stationary phases or chiral mobile phase additives can be applied.

2 Cyclodextrins (CDs)

CDs represent the most frequently used chiral selectors. Native CDs are cyclic oligosaccharides consisting of six (α -CD), seven (β -CD) or eight (γ -CD) glucopyranose units with a truncated cone providing a hydrophobic cavity. Due to the presence of hydroxyl groups the outside of the CD is hydrophilic. Chiral recognition is based on inclusion of the bulky hydrophobic group of the analyte into this hydrophobic cavity of the CD and lateral interactions of the hydroxyl groups at the C-2 and C-3 at the upper rim of the CD, such as hydrogen bonds and dipole-dipole interactions with the analyte. Several neutral and charged CD derivatives have been synthesized. The use of CDs as chiral selectors is the subject of several selective reviews [12, 20, 22].

2.1 Neutral CDs

Various neutral derivatives of CDs such as heptakis-*O*-methyl- β -CD (M- β -CD), heptakis(2,6-di-*O*-methyl)- β -CD (DM- β -CD), heptakis(2,3,6-tri-*O*-methyl)- β -CD (TM- β -CD), hydroxyethyl- β -CD (HE- β -CD), and hydroxypropyl- β -CD (HP- β -CD) have been synthesized and applied to a great variety of compounds [12, 13, 20]. Numerous applications but only a few new developments have been reported in the past three years. Since most of the CD derivatives represent mixtures of different products showing different substitution patterns, separations are often difficult to reproduce. There is a new trend to separate the pure derivatives from mixtures or to synthesize single isomers and to produce selectively substituted derivatives.

Miura *et al.* synthesized selectively methylated [23] and acetylated [24] CDs and evaluated the different products for their ability to resolve Dns-amino acids, naphthalene-sulfonylamino acids and naphthoylamino acids. The authors have shown that there are remarkable differences in selectivity between the methylated and acetylated CDs due to the difference in bulkiness and hydrogen-bonding ability. Also, the position of the methyl or acetyl group

was found to play an important role in chiral recognition ability. Furthermore, the chiral recognition of the different selectors varied for the different analytes depending on their bulkiness. These findings are certainly worthwhile contributions to the developments of chiral selectors and to the clarification of the separation mechanism.

Aturki *et al.* [25] recently introduced a new, uncharged CD derivative, cyanoethylated β -CD, and checked its enantioselectivity with a series of basic and acidic drugs. Acidic drugs were found to be more strongly complexed than basic analytes and lower CD concentrations were required. Interestingly, the authors observed an inversion of migration order for naproxen with this selector compared to trimethyl- β -CD used in previous studies [26]. $^1\text{H-NMR}$ studies indicated a strong interaction between the methyl group and proton of the aromatic moiety of naproxen and the cyanoethylated- β -CD. Chiari *et al.* [27] synthesized a new vinylpyrrolidine- β -CD copolymer by radical copolymerization of vinylpyrrolidone and methacroyl- β -CD. This polymer consists of an alkyl backbone and pendant β -CD units. The chiral recognition ability of this selector was evaluated with a mixture of sympathomimetic drugs. This selector showed significant advantages over native β -CD.

New ethylcarbonate- β - and γ -CDs were introduced by Zerbinati *et al.* [28] and were used for the resolution of racemic dichloroprop herbicides. These new CD derivatives were found to be superior to native CDs, M- β -CD, HP- β -CD and a newly synthesized C-6 capped β -CD. Recently, the synthesis of an interesting compound, an L-Ala-Crown(3)-L-Ala capped β -CD, was reported [29]. The structure of 6^A, 6^D,-dideoxy-*N,N'*-3,6,9-trioxa-undecanoyl-(L)-bis-alanyl- β -CD was confirmed by COSY and ROESY NMR spectra. This new selector was tested by means of the chiral separation of Dns-amino acids.

2.2 Negatively charged CDs

A broad spectrum of negatively charged CDs was investigated and applied to different drug classes, preferentially to basic and neutral drugs. The improved selectivity compared to neutral CDs is mainly attributed to the counter-current mobility. Sulfated β -CDs (S- β -CD), sulfobutyl-(SBE- β -CD) and sulfoethyl ether- β -CD (SEE- β -CD) are the most frequently used charged CDs [12, 20, 21]. The preparation of sulfobutyl- γ -CD was first described by Jung and Francotte [30] and Francotte *et al.* [31]. A reversal of the elution order for several enantiomers was observed on changing from γ -CD to SBE- γ -CD. Rickard *et al.* [32] investigated the influence of the degree of substitution (d.s.) of SBE- β -CDs on the separation and peak shapes using duloxetine as a model compound. From a commercially available SBE- β -CD, fractions of derivatives with dif-

ferent d.s. were isolated and investigated. Other strongly negative charged CD derivatives are sulfated CDs. Commercially available sulfated CDs consist of numerous isomers which differ in their degree and site of substitution. Batch-to-batch variations in composition lead to high variations in mobility and selectivity and so to poor separation reproducibilities. To eliminate these drawbacks, single isomer charged CDs were synthesized.

A family of single isomer β - and γ -CD derivatives was introduced by the group of Vigh [33–35]. These authors prepared derivatives completely sulfated in 6-position and completely substituted on their larger rims with hydrophilic groups (heptakis(2,3-dihydroxy-6-sulfato)- β -CD) [33], moderately hydrophobic groups (heptakis(2,3-diacetyl-6-sulfato)- β -CD) [34] or hydrophobic methyl functional groups (heptakis(2,3-dimethyl-6-sulfato)- β -CD) [35]. More recently, an octakis(2,3-diacetyl-6-sulfato)- γ -CD was synthesized and evaluated [36]. The authors have shown that neutral, basic, zwitterionic and even acidic enantiomers can be separated. As predicted by the charged resolving agent migration, (CHARM) model developed by Williams and Vigh [37], the enantiomeric migration order can be reversed in several cases by increasing the selector concentration.

Highly sulfated CDs with a d.s. of 10, developed by the Beckman company (Palo Alto, CA, USA), were applied to the separation of di- and tripeptides and a broad spectrum of pharmaceuticals. Carboxylfunctional CDs such as carboxymethyl- β -CD (CM- β -CD) [38, 39], carboxyethyl- β -CD (CE- β -CD) [39], and succinyl- β -CD (Succ- β -CD) [39, 40] were introduced and applied to a large variety of compounds [12, 13, 20, 21]. Only a few authors report the use of phosphated CDs [41–43]. Juvancz *et al.* [43] compared the enantioselectivity of α -, β - and γ -CD phosphates for tocainide, metoprolol, and diisopyramide.

2.3 Positively charged CDs

Cationic CDs such as 6-[(3-aminoethyl)amino]-6-deoxy- β -CD [44], 6^A-methylamino- β -CD, 6^A, 6^D-dimethylamino- β -CD [45], a heptasubstituted methylamino- β -CD [46], and mono(6-amino-6-deoxy)- β -CD [47] were the first described cationic CDs to be applied to the chiral separation of different acidic and neutral compounds [12, 13, 20, 21]. O'Keefe *et al.* [48] developed a polycationic CD derivative, heptakis (6-hydroxyethylamino-6-deoxy- β -CD). Using a reversed polarity, acidic compounds such as non-steroidal antiinflammatory drugs (NSAIDs), Dns-amino acids, and phenoxypropionic acid herbicides were resolved adapting the optimal pH for each compound class.

A new hepta-substituted single isomer cationic β -CD (heptakis (6-methoxyethylamine-6-deoxy)- β -CD) was syn-

thesized by Haynes *et al.* [49] and checked for its separation behavior towards NSAIDs and phenoxypropionic acid herbicides. The resolution was found to be strongly dependent on selector concentration and pH. This selector showed improved enantioselectivity for the compounds tested compared to other cationic CDs. The synthesis of several new 6^l-deoxy-6^l-alkyl- and arylamino derivatives of β -CD [50] and their analytical characterization by CE [51] has recently been reported. These new selectors apparently have not yet been applied to chiral separation.

Histamine-modified cationic β -CDs as chiral selectors were recently introduced by Galaverna *et al.* [52] and applied to the enantiomer separation of Dns-amino acids and carboxylic acids as well as hydroxy acids [53]. The authors compared a 6-deoxy-6-*N*-histamino- β -CD, a monosubstituted, positively charged β -CD, bearing a histamine moiety linked to the C-6 of a glucose unit in the upper rim *via* the amino group and a 6-deoxy [4-(2-aminoethyl)imidazolyl]- β -CD bearing the histamine moiety linked to the C-6 *via* the imidazolyl group. Since the latter selector produced only poor resolutions, the authors conclude that the proximity of the positive charge to the cavity plays an important role in chiral recognition. The proposed complexation mechanism, the inclusion of the aromatic ring of the analyte into the cavity and lateral electrostatic interactions between the carboxyl group and the protonated amino group of the CD was confirmed by electrospray ionization-mass spectrometry and two-dimensional nuclear magnetic resonance (2-D NMR) ROESY experiments.

CDs containing quaternary ammonium groups show a pH-independent electrophoretic mobility. Only very low selector concentrations are necessary to resolve acidic enantiomers because of the strong ionic interactions. 2-Hydroxy-3-trimethylammonioethyl- β -CD was investigated by several groups [54–58] and applied to the chiral separation of basic, neutral, and acidic compounds. Bunke and Jira [55, 58] showed that there is a reversal of the electroosmotic flow (EOF) with this selector under the conditions applied. As demonstrated with tropic acid, the elution order of the enantiomers can be reversed by changing from an uncoated to a coated capillary, changing the pH from 5 to 2.5, and reversing the polarity of the voltage. Another quaternary ammonium- β -CD (QA- β -CD) of undefined structure, which is commercially available (CerestarUSA, Hammond, IN, USA), was applied to the chiral separation of various acidic analytes [59–62]. This selector was also shown to be applicable in nonaqueous solvents [61]. The authors investigated formamide (FA), *N*-methylformamide (NMF), methanol, dimethyl sulfoxide and water as solvents. In all solvents the EOF was

reversed above a certain selector concentration. While most of the Dns-amino acids investigated were resolved in all solvents, NSAIDs of the profen type were only resolved in formamide.

2.4 Amphoteric CDs

Lelievre *et al.* [63] synthesized a new zwitterionic selector, mono-(6-glutamylamino-6-deoxy)- β -CD (Glu- β -CD) which was evaluated using neutral, basic, and acidic drugs [63]. The authors investigated this selector at different pH values and observed that enantioselectivity differs depending on the pH. Tanaka and Terabe [64] investigated a commercially available QA- β -CD and an amphoteric β -CD (AM- β -CD). Both CD derivatives were analyzed by CE and MS to determine their composition. QA- β -CD was found to consist of six components having from one to six quaternary ammonium groups. AM- β -CD, which has quaternary ammonium groups and several carboxyl groups, showed many peaks in CE, but the composition could not be completely identified. QA- β -CD was applied to the chiral separation of various carboxylic acids and AM- β -CD to Dns-amino acids. Detailed information and applications of charged CDs can be found in recent reviews [12, 13, 20, 21].

2.5 CDs and nonchiral additives

CDs have often been used in combination with achiral surfactants such as sodium dodecyl sulfate (SDS). This principle was introduced by Terabe *et al.* [65] and called CD-mediated micellar electrokinetic chromatography (CD-MEKC). Negatively charged micelles migrate in the direction opposite to the EOF while uncharged CDs migrate with the same velocity as the EOF. Partition of hydrophobic analytes between the bulk solution, the CD and the micelle phase occurs, causing retention of the analyte. This principle enabled the chiral separation of a broad spectrum of compounds [12, 13, 21]. The enantiomer migration order can be reversed by changing from CD-CZE to CD-MEKC [66–69]. The addition of borate to CDs as a complexing agent was found to be effective [70–73] for the chiral separation of diols. Since resolution was not obtained without borate, the formation of mixed CD-borate diol complexes was assumed. Recently, this principle was applied to the chiral separation of propranolol metabolites with diol structure [74].

3 Carbohydrates

3.1 Neutral polysaccharides

In addition to CDs, linear neutral and charged carbohydrates were also found to be applicable as selectors for chiral separations. Fundamentals and applications up to 1997 are discussed in several reviews [13, 16, 75]. Malto-

dextrins dextrose equivalent (DE) <20 and higher molecular mass dextrans were successfully applied as chiral selectors. D'Hulst and Verbeke [76] found that the chiral recognition ability depends on the DE (percent reducing sugars) and the degree of polymerization (DP, number of saccharide monomers within the oligopolysaccharide chain). They proposed the requirement of α (1 \rightarrow 4) linkage in polysaccharide for chiral recognition, since (1 \rightarrow 6)-linked polysaccharides (dextrans) did not exhibit enantioselectivity for NSAIDs [76, 77]. These findings are in contradiction to later results obtained with other analytes [78, 79].

Chankvetadze *et al.* [80] reported the investigation of water-soluble, native polysaccharides such as amyloses of different DP values and laminaran and pullulan. The authors described also the application of derivatized polysaccharides, methylcellulose and hydroxypropylcellulose as well as carboxymethyl amylose, using (\pm)-1,1'-binaphthyl-2,2'-diyl hydrogen phosphate (BNHP) as a model compound.

Later, Chankvetadze *et al.* [81] investigated a series of noncyclic malto- and oligosaccharides as chiral selectors using BNHP as a model analyte. The authors showed that enantioselectivity is dependent on how the monosaccharide units are linked, and the DP. A change of linkage position between two D-glucose units containing α -linkage from (1 \rightarrow 4) to (1 \rightarrow 6) resulted in a decrease in chiral recognition ability, and a change of (1 \rightarrow 4) linkage to (1 \rightarrow 1) linkage reversed the enantiomeric migration order of BNHP. In the case of disaccharides, a change of α -linkage to β -linkage resulted in disappearance of chiral recognition ability (in the case of maltose and cellobiose) and reversal of migration order for maltotriose and cello-triose.

It should be mentioned that atropisomeric binaphthyl derivatives are very frequently used as model analytes since they are resolved by nearly all chiral selectors. Even monosaccharides have recently been shown to exhibit enantioselectivity for BNHP [82]. Several monosaccharides, such as D-glucose, D-mannose and some of their derivatives were found to be applicable as selectors for the chiral separation of BNHP, while galactose, sorbose, fructose, ribose, arabinose, and xylose failed to resolve BNHP. The authors postulated the following structural requirements for a chiral recognition: (i) oxygen atom at C-1 is in α -configuration; (ii)-OH group at C-4 is configured downward; (iii) the presence of a $-\text{CH}_2\text{OH}$ or $-\text{CH}_3$ group at C-5.

The chiral recognition mechanism for polysaccharide-based selectors is still not completely clear. In the case of dextrans the formation of a helical structure with hydropho-

bic character is assumed to be responsible for the binding of hydrophobic molecules. Lateral binding forces such as hydrogen bondings and dipole-dipole interactions with hydroxy groups of the sugar molecules are to be taken into account [16, 83, 84]. The hypothesis that the chiral recognition ability of dextrans mimics the β -CD cavity [83, 84] was disproved by Chankvetadze *et al.* [85] with the observation that the BNHP- enantiomers showed opposite migration order on maltooligosaccharides and β -CD. Hong *et al.* [86] performed CE complexation studies to elucidate the binding mechanisms between analytes and amyloextrins. Fluorescently tagged amyloextrin oligomers were injected together with model analytes of different structures (S-(+)-ibuprofen, warfarin, ketoprofen and furosemide). The separation patterns of the oligomers were found to be altered by the presence of these pharmaceuticals as buffer additives.

3.2 Charged polysaccharides

Negatively charged polysaccharides such as heparin, chondroitin sulfate C, chondroitin sulfate A, dextran sulfate, λ -carrageenan have been shown to be suitable selectors for the chiral separation of bases [16, 75]. Recently, several new glycosaminoglycans have been investigated as chiral selectors: dermatan sulfate (DS, chondroitin sulfate B) was shown to exhibit enantioselectivity for a broad spectrum of basic drugs including β -sympathomimetics, β -blockers, antihypertensives, antimalarials, *etc.* [87]. DS is a complex, polydispersed, sulfated polysaccharide consisting of (1 \rightarrow 4)-O-(α -idopyranosyluronic acid)-(1 \rightarrow 3)-O-(2-acetamido-2-deoxy- β -D-galactopyranosyl-4-sulfate) disaccharide units. Semisynthetic chondroitins on the basis of oversulfated galactosaminoglycans were recently prepared by the same group [88]

and successfully applied to the chiral separation of basic drugs belonging to different classes.

Tsukamoto *et al.* [89] introduced two new glycosaminoglycans, a fucose-containing glycosaminoglycan (FGAG) and depolymerized holothurian glycosaminoglycan (DHG) and applied them to the chiral separation of a variety of basic drugs. These glycosaminoglycans were found to be complementary to other selectors. Tolperison and eperison, for example, which could not be separated with α - and β -CD or heparin, were separated with FGAG and DHG. The authors have shown that the resolution depends on the molecular mass and the selector concentration. Armstrong's group [90] recently investigated the use of pentosan polysulfate (PPS) as a chiral selector. PPS is a semisynthetic sulfated glycosaminoglycan composed of D-xylopyranose units connected through β -(1 \rightarrow 4)-linkage. The applicability of this chiral selector was demonstrated by the chiral separation of tryptophan derivatives and several drugs. Figure 1 gives examples for the application of this new chiral selector.

Phinney *et al.* [91] investigated citrus pectins (polygalacturonic acid sodium and potassium salts and esterified pectins) as chiral selectors for the separation of basic drugs including antihistamines and antimalarial drugs as well as broncho- and vasodilators. The authors showed that esterification of the galacturonic acid has a deleterious effect on enantioresolution. Nishi *et al.* [92] introduced several positively charged polysaccharides. Diethylaminoethyl dextran, and the aminoglycoside antibiotics streptomycin sulfate, kanamycin sulfate and fradiomycin sulfate were applied for the resolution of some acidic analytes. Since then, there have been no further reports on the use of positively charged polysaccharides.

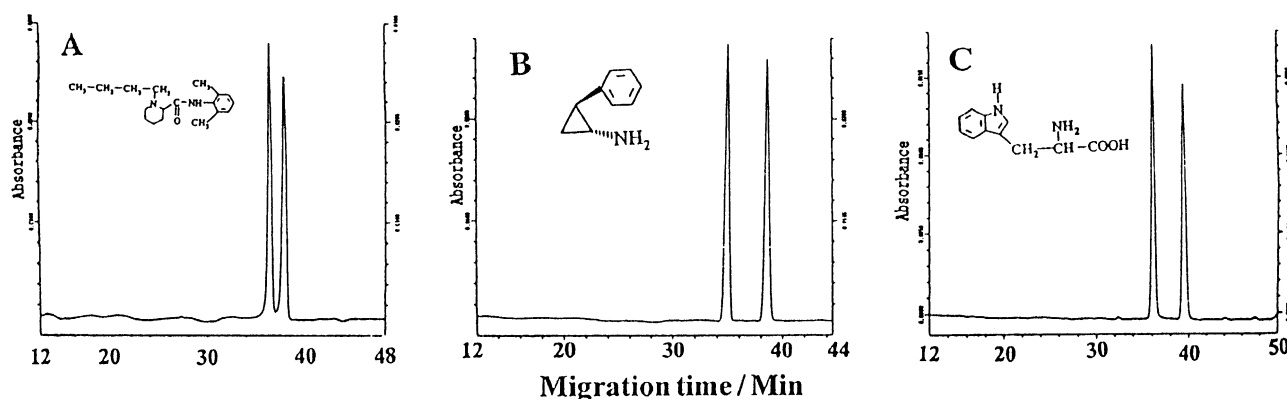


Figure 1. Separation of racemic (A) bupivacaine, (B) *trans*-2-phenylcyclopropylamine and (C) tryptophan. Running solution, 1% w/w pentosan polysulfate in 10 mM phosphate buffer (pH 2) containing (A) 20%, (B) 30%, and (C) 50% v/v methanol. Voltage applied, 28 kV; capillary, 57 cm (50 cm to the detector) \times 75 μ m ID; temperature, 23°C; detection wavelength, 214 nm. Reprinted from [90], with permission.

4 Chiral crown ethers

Crown ethers are cyclic polyethers which form host-guest complexes with earth metal ions and primary ammonium cations. Chiral crown ethers can stereoselectively include compounds containing primary amino groups. The only chiral crown ether used in CE to date is 18-crown-6-tetracarboxylic acid, (18C6H4) which was synthesized by Behr *et al.* [93]. According to Kuhn *et al.* [94], the primary interactions for complexation are hydrogen bonds between the three amine hydrogens and the oxygens of the macrocyclic ether in a tripod arrangement. In addition, the carboxylic acid groups are arranged perpendicular to the plane of the macrocyclic ring, forming a chiral barrier, which divides the space available for the substituents at the chiral center into two domains. Thus, two diastereomeric inclusion complexes are formed. Furthermore, ionic-, dipole-dipole interactions or hydrogen bonds between the carboxylic groups and polar groups of the analytes may act as additional supporting interactions.

This selector was introduced by Kuhn *et al.* [95] for the chiral separation of amino acids by CE and found application, among others, in the chiral separation of sympathomimetics [94, 96], dipeptides [97, 98], various amino acid derivatives [99, 100], and different drugs containing primary amino groups [101]. Mori *et al.* [102] described the chiral separation of various drugs using 18C6H4 in non-aqueous medium. Comprehensive surveys of applications of chiral crown ethers to various compounds have recently been provided by Kuhn [103] and Verleysen and Sandra [21].

Verleysen *et al.* [99] tested 18-crown-6-tetracarboxamide, an intermediate in the synthesis of 18C6H4, for its ability to resolve primary amines by CE; however, this compound did not show any stereoselectivity. Tanaka *et al.* [104] recently described a partial filling technique using 18C6H4 in combination with MS detection to prevent entry of the nonvolatile selector into the nozzle of the CE-MS interface and the orifice plate. This technique was performed with racemic 3-aminopyrrolidine and α -amino- ϵ -caprolactam, which absorb UV only weakly. The combination of 18C6H4 and achiral crown ethers with CDs is discussed in Section 12.

5 Chiral calixarenes

Calixarenes represent a new type of chiral selectors. They are macrocyclic compounds consisting of benzene rings linked by methylene groups forming a hydrophobic cavity which is able to form host-guest complexes. Peña *et al.* [105, 106] synthesized water-soluble (*N*-L-alanine)calix[4]arene and (*N*-L-valine)calix[4]arenes.

These calixarenes possess four amino acid residues on the lower rim which permit chiral recognition. The authors resolved BNHP, (\pm)-1,1'-bi-2-naphthol (BINOL) and (\pm)-1,1'-binaphthyl-2,2'-diamine (BNA) as model compounds with these chiral calixarenes. Grady *et al.* [107] synthesized a (*S*)-di-naphthylprolinol calix[4]arene and coated the wall of the capillary with it for its hydrophobic properties. The authors demonstrated the applicability of this approach for chiral separations using 2-phenylglycinol as a model compound. Fluorescence quenching experiments with Stern-Volmer plots confirm that the (*R*)-enantiomer of phenylglycinol interacts more strongly with the calixarene.

6 Macrocyclic antibiotics

Macrocyclic antibiotics as chiral selectors were introduced by Armstrong *et al.* [108]. They have several asymmetric centers and many functional groups, allowing multiple interactions with the analytes. In addition to ionic interactions, hydrogen bonding, dipole-dipole, π - π , hydrophobic interactions and steric repulsion are assumed to take effect [109]. Macrocyclic antibiotics possess hydrophobic pockets which can include hydrophobic moieties; due to the presence of pendant polar arms, hydrogen bonds can be formed.

Three classes of antibiotics have been introduced as chiral selectors: Ansamycins such as rifamycin B, rifamycin SV; the glycopeptides vancomycin, ristocetin and teicoplanin and the aminoglycoside antibiotics streptomycin, fradiomycin and kanamycin. A comprehensive description of the properties of these selectors and their applications to the chiral separation of a broad spectrum of compounds is given in several specialized reviews [19, 110, 111]. While rifamycin B showed enantioselectivity for basic compounds, rifamycin SV and the glycopeptide antibiotics were found to be suitable for the chiral separation of acidic compounds.

Since these macrocyclic antibiotics contain aromatic moieties and have a strong UV absorption up to 250 nm, detection is only possible at wavelengths higher than 250 nm. Alternatively, indirect detection can be used. To overcome the detection problems, countercurrent processes have been applied [112, 113]. In this approach, a coated capillary was used to suppress the EOF and a suitable pH provided the selector (vancomycin or ristocetin A) and the analytes with opposite charges. Thus, the positively charged selector moves to the cathode, clearing the detection window, and the analytes can be detected without interferences at the anode. Fanali's group [114–116] used a partial filling method together with the countercurrent mode using vancomycin or teico-

planin as chiral selectors. Countercurrent approaches were also used to allow an interference-free coupling with MS using vancomycin as a chiral selector [117, 118].

Subsequently, some new macrocyclic antibiotics of the glycopeptide type were investigated: Strega *et al.* [119] introduced the glycopeptide antibiotic A 82846B, which was also investigated in countercurrent mode for the chiral separation of profens [120]. LY307599, a derivative of A 82846B, which differs from the latter compound by an additional biphenyl moiety, was evaluated using flurbiprofen [121]. Actaplanin A [122] represents a further member of this family which also found application to the chiral separation of NSAIDs of the profen type.

Avoparcin, a new glycopeptide antibiotic was recently introduced by Armstrong's group [123]. It consists of a mixture of several structurally similar glycopeptide analogues of which α -avoparcin and β -avoparcin are the major components. The authors evaluated the chiral recognition ability of this selector using Dns-amino acids and NSAIDs and compared the results with those obtained with ristocetin A, teicoplanin and vancomycin. Fanali *et al.* [124] evaluated a new antibiotic, Hepta-tyr (MDL 63,246). Hepta-tyr is a semisynthetic small glycopeptide antibiotic, which belongs to the teicoplanin family. Using the partial filling method, this selector was applied to the chiral separation of acidic compounds, among them NSAIDs, anticoagulants and herbicides of the phenoxypropionic acid type using a boric acid-acetic acid-phosphoric acid buffer, pH 5 / acetonitrile mixture as electrolyte.

A further member of the glycopeptide family, A 35512B, was recently investigated by Risley *et al.* [125]. Its good water solubility allows the use of aqueous buffer solutions as mobile phases. The enantioselectivity of this selector was checked using Dns-amino acids. Increasing selector concentration was shown to increase resolution. The influence of pH on resolution was investigated over a range from 6 to 8. In general, the resolution increased with decreasing pH. Baseline resolution for eleven out of thirteen Dns-amino acids tested could be achieved using just an aqueous phosphate buffer, whereas 2-methoxyethanol had to be added to resolve Dns-methionine and Dns-threonine.

To reduce migration time, Kang *et al.* [126] added hexadimethrine bromide, a polycationic polymer, to the run buffer to reverse the EOF. The cationic polymer adsorbs to the capillary wall and adsorption of vancomycin is avoided. The acidic analytes migrate in a co-EOF mode, thereby drastically reducing the migration time. The applicability of this approach is demonstrated with the chiral separation of 9-fluorenylmethoxycarbonyl (Fmoc)-

amino acids and ketoprofen. Proof of the hypothesis that the enantiorecognition of macrocyclic antibiotics takes place at the D-Ala-D-Ala binding site, located in the aglycon core, was given by Carotti *et al.* [127]. Teicoplanin was applied in a mixture with D-Ala-D-Ala or L-Ala-L-Ala in the electrolyte using *p*-methoxymandelic acid as a model analyte. When D-Ala-D-Ala was present, no chiral resolution was obtained, whereas with L-Ala-L-Ala there was no significant change in resolution.

7 Proteins

The well-known phenomenon that drugs bind stereoselectively to proteins led to investigations to use proteins as chiral selectors. Several proteins have been successfully applied in the form of chiral stationary phases in HPLC. A great variety of proteins, such as bovine serum albumin, human serum albumin, α_1 -acid glycoprotein, avidin, conalbumin, cellulase, ovomucoid, cellobiohydrolase and casein were used as chiral selectors in CE for a broad spectrum of compounds. For literature covering applications up to the year 1997 and detailed information the authors recommend specialized reviews [14, 128, 129]. Proteins can be positively or negatively charged depending on the pH applied. Their charges give them electrophoretic mobility and they can be used for the separation of basic and acidic analytes.

Fanali *et al.* [130] reported on the use of pepsin as a chiral selector using a partial filling technique. A polyacrylamide-coated capillary was used to suppress the EOF and to avoid adsorption of the protein on the capillary wall. The authors evaluated this selector using several β -blockers and some other basic drugs. It was shown that with increasing selector concentration, resolution generally increased, and that the pH investigated in the range between 4 and 7 had to be adapted individually for each compound. The addition of organic modifiers resulted in a decrease in resolution. Tanaka and Terabe [131] studied the enantioselectivity of egg white avidin, succinylated avidin and streptavidin using a partial filling technique. While the basic avidin was found to be useful for the chiral separation of acidic analytes, succinylated avidin showed enantioselectivity for basic analytes. Streptavidin, a neutral nonglycosylated protein, showed enantioselectivity for both acidic and basic analytes. The authors showed that chiral recognition ability was lost when biotin was added as it forms a strong complex with avidin in which the stereoselective binding sites are blocked (Fig. 2).

De Lorenzi *et al.* [132, 133] evaluated purified quail egg white riboflavin binding protein as a chiral selector for HPLC and CE. Riboflavin-binding proteins are acidic proteins with a molecular weight of about 36 000 containing

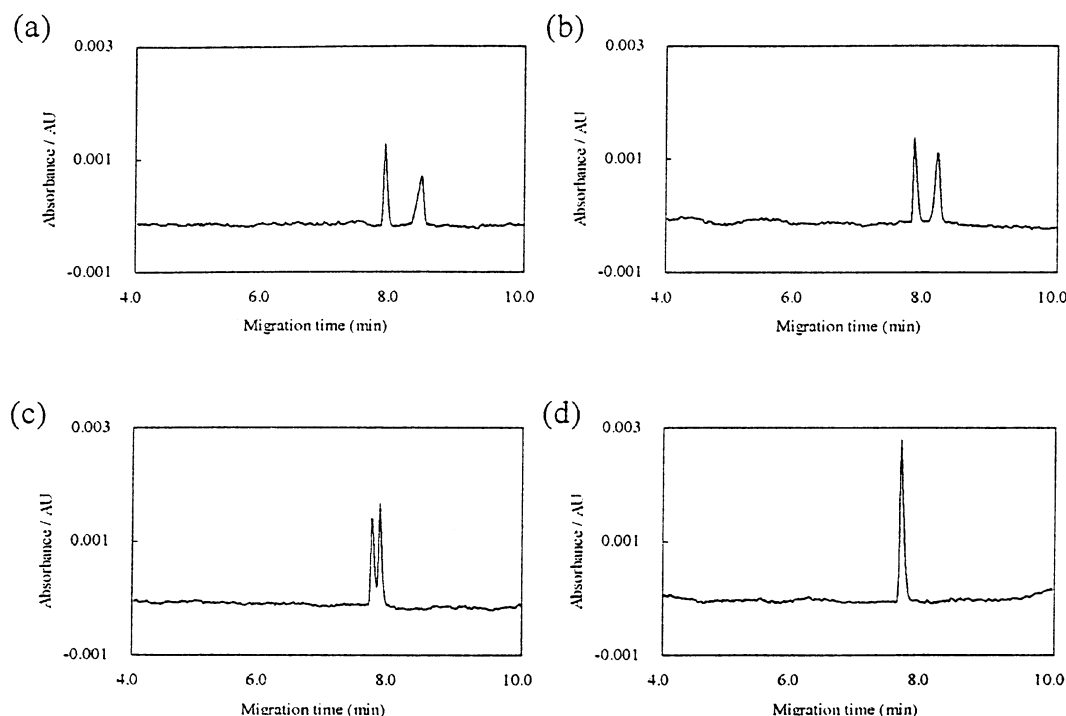


Figure 2. Effect of biotin binding to avidin on enantiomer separation of racemic 4-fluoromandelic acid. Conditions: capillary, 36 cm \times 50 μ m ID polyacrylamide-coated; running buffer, 50 mM phosphate buffer (pH 4.0); separation solution, 100 μ M avidin in running buffer containing (a) 0 μ M, (b) 100 μ M, (c) 200 μ M, (d) 400 μ M D-biotin (zone injection, 6.9 kPa \times 190 s); applied voltage, -12 kV; sample injection, 6.9 kPa \times 2s; capillary temperature, 25°C, detection wavelength, 210 nm. Reprinted from [131], with permission.

about 14% carbohydrate. Complete and partial filling techniques were compared [133] using several basic drugs such as oxazepam, oxprenolol, prilocaine, bupivacaine, *etc.*, as model compounds. Mano *et al.* [134] recently reported the use of native flavoprotein isolated from chicken egg white and chemically modified flavoproteins as chiral selectors. NSAIDs of the profen type, oxprenolol, proglumide and aminoglutethimide, were used to check the chiral recognition ability. The chiral recognition region on the flavoprotein is assumed to consist of an α -helix structure. Studies with the chemically modified flavoproteins led to the conclusion that there are ionic interactions between an amino group and a carboxyl group of the protein and the carboxyl group of the model compound ketoprofen and π - π interaction of the tryptophan moiety of the protein and the aromatic of the analyte.

8 Ligand-exchange CE

The first application of the principle of ligand-exchange in CE was reported by Zare's group. They used L-histidine- [135] or aspartam/Cu(II) [136] complexes for the chiral separation of Dns-amino acids. Desiderio *et al.* [137] re-

solved hydroxy acids using L-Pro-, L-Hypro- or aspartam / Cu(II) complexes. Sootornniyomkij *et al.* [138] investigated the role of the metal ion on the resolution of Dns-amino acids using aspartame as chiral selector. The first approach for direct separation of underivatized amino acids using L-Pro or L-Hypro / Cu(II) complexes as chiral selectors was published by Schmid and Gübitz [139]. Using MEKC with SDS added to the electrolyte led to a reversal of the enantiomer migration order (EMO). This is probably caused by hydrophobic and electrostatic interactions with the negatively charged micelles for which the D-enantiomer is more easily accessible.

Chen *et al.* [140] applied a similar principle to the separation of the positional isomers and enantiomers of fluoro-phenylalanine and tyrosine using L-Hypro with or without SDS. A reversal of the EMO was also observed by these authors when SDS above a certain concentration was added to the electrolyte. The same group [141] reported the separation of sixteen positional and optical isomers of tryptophan derivatives checking SDS, *n*-decyl sulfate (SDeS) and *n*-tetradecyl sulfate (STS) as micelle-forming surfactants. In another paper [142], the mechanism for the flow reversal observed by addition of SDS, SDeS and

STS is discussed and the influence of other surfactants such as Tween-20 and cetyltrimethylammonium bromide (CTAB) as well as organic solvents on the separation is studied. Tween-20 was found to improve resolution at the expense of retention time; the addition of CTAB resulted in a change of the direction of the EOF, but did not improve resolution. Organic modifiers did not inverse the EMO but caused a decrease in resolution.

Recently, this group [143] published a method for the determination of the critical micelle concentration (CMC) on the basis of ligand-exchange MEKC. This approach is based on the above-mentioned observation that the point of the reversal of the EMO is identical to the CMC. These authors did not observe a reversal of the EMO in the case of hydroxy acids when SDS was added using *trans*-L-Hypro-Cu(II) as selector [144]; this is in contrast to amino acids. A reversal, however, was obtained when the cationic surfactant CTAB was used instead of SDS due to a reversal of the EOF. Interestingly, a reversal of the EMO was also observed when *trans*-L-Hypro was changed for *cis*-L-Hypro. In recent studies, this group [145] showed that the EMO for amino acids also depends on the type of ligand used. While L-Pro and *trans*-L-Hypro showed the same EMO, there was a reversal of the EMO with *cis*-L-Hypro, probably due to the fact that *cis*-L-Hypro represents a tridentate ligand. As was expected, the EMO could also be reversed by changing the chirality of the selector.

Yuan *et al.* [146] reported the use of L-arginine / Cu(II) for the resolution of Dns-amino acids. The resolution was lost when Cu(II) was replaced by Ni(II), Co(II) or Zn(II). When L-Arg was substituted by L-Glu, L-Ala and L-Asp, also no resolution was obtained.

N-alkyl-hydroxyproline such as *N*-(2-hydroxyoctyl)- and *N*-(2-hydroxypropyl)-L-4-hydroxyproline derivatives have recently been synthesized and applied as Cu(II) complexes to the chiral separation of underivatized aliphatic and aromatic amino acids and dipeptides [147, 148]. Compared to L-Hypro, these selectors showed improved resolution with significantly lower selector concentrations. *N*-(2-hydroxyoctyl)-L-4-hydroxyproline was also shown to be applicable for the chiral resolution of sympathomimetic drugs [149] as well as hydroxy acids and β -blockers [150]. Figure 3 shows the chiral resolution of ephedrine, octopamine and orciprenaline. Under these conditions, orciprenaline showed only partial resolution.

More recently, Karbaum and Jira [151] have shown that ligand-exchange CE (LECE) is also possible in nonaqueous solvents. The authors used L-proline / Cu(II) in 25 mM ammonium acetate / 1 M acetic acid in methanol to resolve several aromatic amino acids. The use of L-isoleucine instead of L-proline decreased enantioselectivity

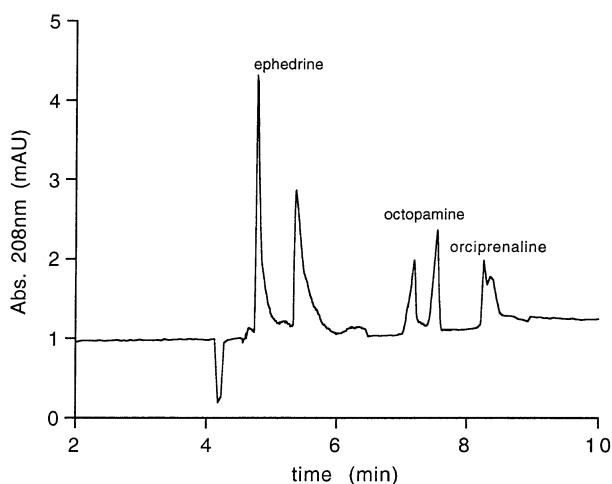


Figure 3. Electropherogram of the chiral separation of (±)-ephedrine, (±)-octopamine and (±)-orciprenaline. Conditions: capillary, 70 cm (26 cm to the detector) \times 50 μ m ID; electrolyte, 10 mM HO-L-Hypro, 5 mM Cu(II) at pH 12; voltage applied, 15 kV; temperature, 25°C; detection wavelength, 208 nm; injection: 30 mbar \times 6 s. Reprinted from [149], with permission.

except for kynurenine and histidine, which could not be separated with L-Pro. The preparation of monolithic phases for LE-CEC [152] is discussed in Section 14.3.

9 Chiral ion-pairing reagents

Although no reports exist to date of successful application of chiral ion-pairing reagents using aqueous BGEs, several ion-pairing reagents have been applied successfully in nonaqueous CE. Obviously, water interferes with the intermolecular interactions responsible for chiral recognition and largely suppresses the formation of intermolecular hydrogen bonds. Exceptions are the use of chiral and achiral counterions as supporting agents for separations with CDs in dual systems [153] (see Section 12). The first ion-pairing reagent reported for CE separations was (+)-*S*-camphor-10-sulfonic acid, which was applied for the chiral separation of basic compounds [154]. To date, this approach has only been successful with compounds with a β -amino alcohol structure. Acetic acid in acetonitrile containing Tween-20 was used as electrolyte. In addition to ionic interactions, dipole-dipole interactions and hydrogen bondings are responsible for chiral recognition. Tween is assumed to act as a hydrophobic pseudostationary phase. Stalcup and Gahm [155] used quinine as an ion-pairing reagent in nonaqueous medium for the chiral resolution of acidic compounds using acetic acid ammonium acetate methanol as BGE.

Lämmerhofer and Lindner [156] and Piette *et al.* [157] investigated quinine and *tert*-butyl carbamoylated quinine as chiral ion-pairing reagents for the enantioseparation of

N-protected amino acids testing ammonium acetate in methanol and ammonia-octanoic acid in an ethanol-methanol mixture as nonaqueous BGEs. Recently, Piette *et al.* [158] screened several cinchona alkaloids and derivatives for their ability to act as chiral counterions for the separation of different *N*-protected amino acids in nonaqueous systems. The authors observed rather poor enantioselectivity when using the natural cinchona alkaloids quinine, quinidine, cinchonine, and cinchonidine. Significantly higher resolution values were obtained with *tert*-butyl carbamoylated quinine, *tert*-butyl carbamoylated quinidine, dinitrophenyl carbamoylated quinine and cyclohexyl carbamoylated quinine as counterions and ethanol-methanol mixtures with ammonia-octanoic acid as BGEs. The best enantioseparations were achieved for Dns-amino acids. Resolution factors up to 78 were observed. The enantioselectivities and the enantiomer migration orders were found to be strongly dependent on the type of selector. In addition to the primary ionic interactions, hydrogen bonding, dipole-dipole, charge transfer (π - π), hydrophobic and steric interactions are to be taken into account.

10 Chiral surfactants

The principle of MEKC using chiral surfactants as selectors was introduced by Terabe *et al.* [159]. Surfactants are amphiphilic molecules composed of a polar head group and a hydrophobic tail. Above a certain concentration (CMC) micelles are formed. These micelles are used as pseudostationary phases in MEKC. The chiral separation of analytes is based on their partition coefficients between the chiral micelle phase and the electrolyte bulk phase.

Different classes of surfactants were applied as chiral selectors: bile salts, saponines, long-chain *N*-alkyl-L-amino acids and *N*-alkanoyl-L-amino acids, *N*-dodecoxy-carbonyl amino acids, alkylglycoside surfactants and polymeric amino acid-based surfactants [160, 161]. New carbamate-type surfactants were synthesized by Ding and Fritz [162] from amino acids (L-Leu, L-Val, L-Ile, L-Ser) and alkylchloroformates with chain lengths of C4 to C11. These new selectors were evaluated using propranolol, atenolol, ketamine, laudanosine, nefopam, benzoin and hydrobenzoin as model compounds. The enantioselectivity was found to depend on the amino acids used and the chain length. A significant improvement in resolution was observed when a dual selector system consisting of the carbamate surfactant and a sulfonated- β -CD is used.

Recently, polymeric dipeptide surfactants have been introduced [163–165]. The monomers were synthesized by coupling the *N*-hydroxysuccinimide ester of 10-undecylenic acid to the dipeptide. A survey of the evaluation of

different dipeptide polymeric surfactants was recently published by Haddadian *et al.* [166]. The authors have shown that the order of the amino acids in the dipeptide has a marked influence on the enantioselectivity. The applicability of a variety of dipeptide polymeric surfactants for the chiral separation of acidic, basic and neutral analytes was demonstrated. A significant improvement in resolution compared to polymeric amino acid-based surfactants, previously published [161, 167], was observed. The sites of interaction of the analytes with the polar head group of the surfactant is dependent on the depth of penetration of the analyte into the hydrophobic micellar core of the dipeptide chiral pseudostationary phase (CPSP). The depth of penetration is determined by the hydrophobicity of the analytes, as well as by electrostatic interactions of the enantiomers with the surfactant. Since the *N*-terminal amino acids are located close to the hydrophobic core and the *C*-terminal amino acids at the interface of the micelle, hydrophobic enantiomers which penetrate more deeply into the hydrophobic core will interact mainly with the *N*-terminal amino acids. Hydrophilic and cationic enantiomers also interact mostly with the *C*-terminal amino acids. Moderately hydrophobic enantiomers can interact with both amino acids of the CPSP. The authors demonstrated that the enantiomeric migration order strongly depends on the amino acid sequence in the dipeptide CPSP.

Two isomeric D-glucopyranoside-based surfactants were synthesized by Tickle *et al.* [168]. To better understand the possible mechanism of chiral discrimination, the authors calculated the minimum energy conformations of micelles produced by these surfactants. They also investigated the microstructures by transmission electron microscopy. Ju and El Rassi [169, 170] recently presented new chiral glycoside surfactants, cyclohexyl-butyl- β -D-maltoside, cyclohexyl-pentyl- β -D-maltoside and cyclohexyl-pentyl- β -D-maltoside. These cyclohexyl-alkyl- β -D-maltosides have a chiral maltose polar head group and a cyclohexyl-alkyl hydrophobic tail. They form neutral micelles in aqueous media. Since the neutral micelles migrate at the velocity of the EOF, they are only useful for the enantiomer separation of charged enantiomers. These chiral selectors were evaluated using Dns-amino acids, dinitrophenyl (DNP)-amino acids, tryptophan derivatives, and BNHP.

Recently, two new amphiphilic aminosaccharide derivatives, namely 2-carboxy-1-(carboxymethyl)ethyl-6-*O*-[4-carboxy-2*R*-tetradecanoylamino]butanoyl]-2-deoxy-3-*O*-tetradecanoyl-2-tetradecanoyl-amino- α -D-glucopyranoside and 2-carboxy-1-(carboxymethyl)ethyl-6-*O*-[3-carboxy-3*S*-tetradecanoyloxypropanoyl]-2-deoxy-3-*O*-tetradecanoyl-2-tetradecanoylamino- α -D-glucopyranoside were synthe-

sized by Horimai *et al.* [171]. These negatively charged selectors were applied above their CMC in borate buffer, pH 9.5, for electrokinetic separation of Dns-amino acids and new quinolon antibacterial agents. The authors found that increase of the selector concentration or BGE concentration led to an increase in enantioselectivity. The decrease of electrostatic repulsion and the increase of hydrophobic interaction between the negatively charged pseudostationary phase and the analyte are assumed to be important driving forces for the chiral discrimination.

Bunke *et al.* [172] investigated (–)-*N*-dodecyl-*N*-methylphedrinium bromide (DMEB) as selector for the chiral separation of the atropisomeric methaqualone. Since DMEB is a hydrophobic quaternary ammonium salt, it interacts with the capillary wall of the uncoated capillary and the EOF is reversed. Resolution of methaqualone was only obtained with high concentrations of DMEB. Methaqualone is included in the positively charged micelle and due to the higher mobility of the EOF moves in the direction of the EOF to the anode. When a neutrally coated capillary was used, the analyte moved to the cathode due to the charge of the micelles. An enantioseparation was also observed in this case, but with a lower separation factor.

11 Nonaqueous medium

Nonaqueous CE (NACE) is becoming increasingly popular. Recently, Karbaum and Jira [173] checked several solvents for their suitability for NACE and discussed its advantages. Nonaqueous solvents show several advantages regarding solubility of chiral selectors or samples and reduce unwanted interactions with the capillary wall. An increase in selectivity can often be observed in nonaqueous solvents. Different forms of chemical equilibria in aqueous and nonaqueous systems can lead to different selectivities. Weak interactions which are disrupted by water can become effective in nonaqueous systems. One example for that is the enantiomer separation by ion-pair formation. In nonaqueous solvents, less Joule heating is produced and since higher voltage can be applied, retention times are shorter. Furthermore, nonaqueous solvents are better compatible with CE-MS coupling than aqueous BGEs. First applications of nonaqueous solvents in chiral CE separations were reported by Valkó *et al.* [174] and Wang and Khaledi [175] using CDs in FA, DMF and NMF as solvents.

Wang and Khaledi [176] have recently reviewed the use of NACE for chiral separations. CDs are the most frequently used chiral selectors in nonaqueous solvents. In addition to neutral CDs, charged CDs were used in NACE. Only a few examples will be mentioned here: com-

parison of the separation of basic compounds in aqueous and nonaqueous systems using S- β -CD in FA showed that there is a significant reduction of band broadening in nonaqueous medium [177]. Vincent and Vigh [178] used the single isomer heptakis(2,3-diacetyl-6-sulfato)- β -CD in pure methanol for the chiral separation of a great variety of basic drugs with remarkable efficiency. The use of a QA- β -CD in pure organic solvents for the chiral separation of amino acids derivatives and some profens was reported by Wang and Khaledi [179].

Mori *et al.* [102] described the application of a chiral crown ether (18C6H4) to the chiral separation of several compounds with primary amino groups in FA. The use of chiral ion-pair reagents such as (+)-*S*-camphorsulfonate [154], quinine [157], and quinine derivatives [157, 158] is discussed in Section 9. In a recent paper, Karbaum and Jira [151] demonstrated the applicability of the principle of chiral ligand-exchange in NACE using L-Pro / Cu(II) in ammonium acetate / acetic acid / methanol. This new approach found application to the separation of underivatized amino acids and drugs with amino alcohol structure. Nonaqueous CEC (NACEC) is a recent trend. Krause *et al.* [180] prepared a helically chiral poly(diphenyl-2-pyridylmethyl) methacrylate coated on aminopropyl-silanized silica. Chiral separation of neutral compounds was carried out in pure methanol. Tobler *et al.* [181] reported the application of a chiral stationary phase (CSP) based on *tert*-butylcarbamoylquinine immobilized on silica gel to NACEC using acetic acid/triethylamine in acetonitrile/methanol as mobile phase (see also Section 14.2).

12 Dual selector systems

The combination of two chiral selectors was found to improve resolution in many cases. Sometimes no separation is obtained using only one of the chiral selectors. The dual selector systems most frequently used are different CDs. The combination of neutral native and CD derivatives significantly enhanced resolution in many cases. Often neutral and charged CDs were combined and improved or even enabled resolution. Since specialized reviews [182, 183] deal with the use of dual CD systems, this will not be detailed here.

The combination of CDs with chiral surfactants such as bile salts [184–187], decanoyl-*N*-methyl glucanoid [188] and poly(sodium-*N*-undecenyl-D-valinate) [189] was found to improve resolution. Synergistic effects were observed when 18C6H4 was used in combination with neutral CDs [190–192]. Recently, Verleysen and Sandra [193] investigated the use of 18C6H4 in combination with negatively charged CDs and the application of a partial filling method [193]. The behavior of primary amines using

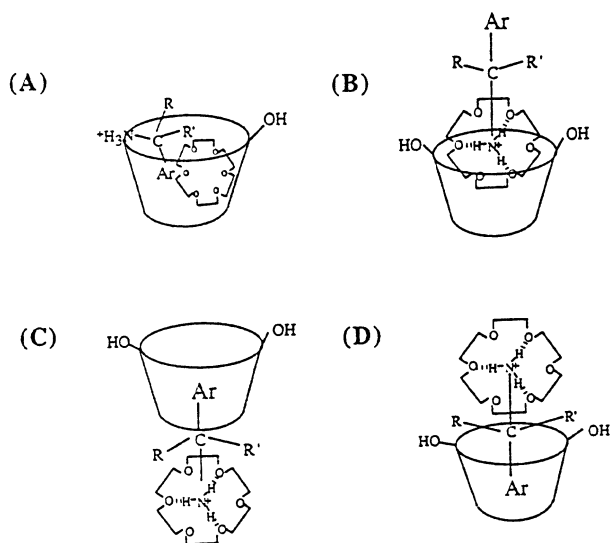


Figure 4. Some possible “three-body” complexes between a hypothetical chiral amine, a CD and 18-crown-6. “D” is thought to be the most important and dominant complex. Reprinted from [198], with permission.

coated and uncoated capillaries at high and low pH was investigated. The synergistic effect of the selector combination is explained and a possible separation mechanism discussed.

The addition of nonchiral crown ethers to CDs was also found to have supporting effects on chiral separations

[194–197]. The authors showed that several primary amines, which could not be separated by CDs alone, were separated after addition of 18-crown-6. Similar investigations were reported by Armstrong *et al.* [198], who discussed several possibilities of “three body” complexes between an amine, a CD, and 18-crown-6 (Fig. 4). The chiral recognition mechanism is explicated by showing the solution equilibria steps (Fig. 5).

Bunke *et al.* [199] observed that the chiral resolution of the drug cyclodrine is only possible with a mixture of β -CD and (+)-*S*-camphor-10-sulfonic acid (CSA). Neither with β -CD nor with CSA alone was resolution obtained. Jira *et al.* [200] investigated several chiral and achiral ion-pairing reagents regarding their supporting effect on the chiral separation using CDs. The authors found out that a significant improvement in resolution is observed regardless of whether the counterion is chiral or not. The influence of (+)- or (–)-camphorsulfonic acid, alkylsulfonic acids and alkanolic acids of different chain length as well as sodium cyclamate on the chiral resolution of basic compounds using different neutral CDs was investigated. On the other hand, basic counterions such as quinine and (*S*)-hyoscyamine were found to improve the resolution of acid compounds using CDs. The combination of the chiral ligand-exchange principle with host-guest complexation was realized by Horimai *et al.* [201] using a dual selector system consisting of γ -CD and Zn(II)-*D*-phenylalanine. This dual selector system was applied to the chiral separation of new quinolone drugs.

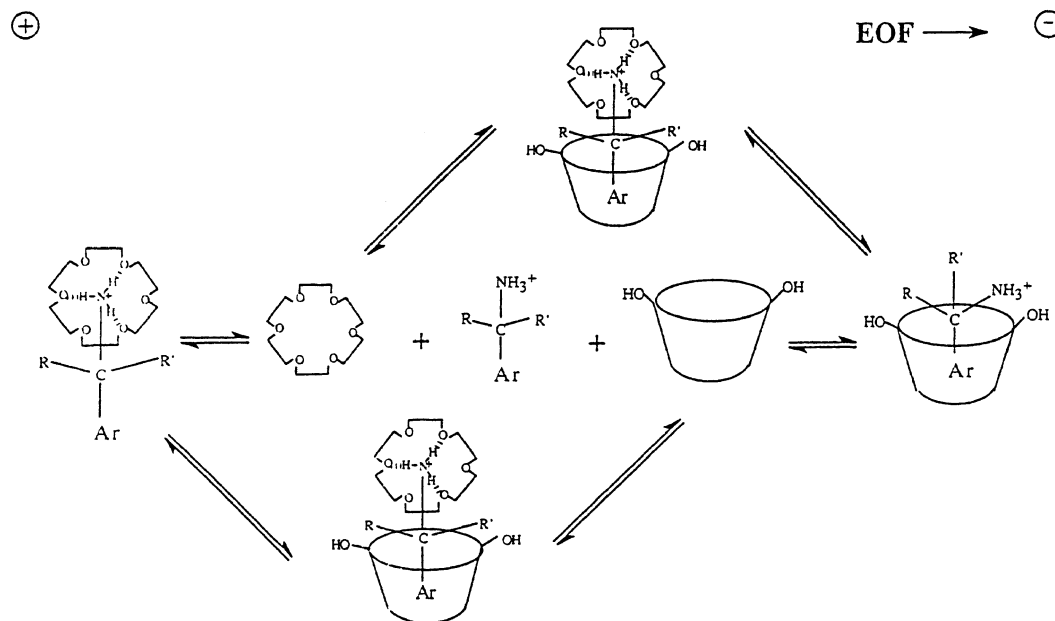


Figure 5. Schematic showing the most important solution equilibria steps in the formation of the “three-body” complex. Reprinted from [198], with permission.

13 Diverse selectors

(+)-(5*R*,8*S*,10*R*)-1-allylterguride, a semisynthetic product, derived from ergot alkaloids, was shown to be a suitable chiral selector for the separation of hydroxy acids [202] and herbicidal enantiomers [203]. A further publication by this group [204] deals with the determination of the formation constant of 1-allyl terguide with mandelic acid by measuring the effective mobility of acidic analytes at varying concentrations. The use of poly-terguide in CEC [205] is discussed in Section 14.1. Nair *et al.* [206] reported the use of D(+)-tubocurarine chloride as a chiral selector. This compound, a macrocyclic bis(benzylisoquinoline) alkaloid, was evaluated by means of the chiral separation of 18 carboxylic acids using methanol / phosphate buffer as electrolyte. Two groups [207, 208] reported the use of cyclohexapeptides prepared by combinatorial synthesis for chiral separations. Several cyclic peptide libraries were prepared and checked for their ability to resolve DNP amino acids.

14 CEC

CEC represents a hybrid method between CE and HPLC making use of the efficiency of CE and the selectivity of stationary phases. Different variants of these recent techniques have been developed for chiral separations. In chiral open tubular capillary electrochromatography (OT-CEC), the chiral selector is covalently attached or coated on the inner surface of a capillary. In contrast, in packed CEC (P-CEC) either an achiral stationary phase in combination with a chiral mobile phase or a CSP can be used. A new alternative to silica-based packed capillaries is the use of monolithic chiral stationary phases prepared by *in situ* polymerization procedures.

14.1 Open tubular capillaries

Several specialized reviews report the use of CEC for chiral separations [209–211]. The principle of chiral CEC was introduced by Schurig's group [212–216]. Permethylated β -CD was covalently linked *via* an octamethylene spacer to a dimethylpolysiloxane and coated on the capillary wall (Chirasil-Dex). These phases were applied to the chiral separation of a broad spectrum of drugs. The possibility of unified chromatography using the same capillary coated with Chirasil-Dex for GC, capillary HPLC, supercritical fluid chromatography (SFC) and CEC was demonstrated [216]. Armstrong *et al.* [217] published a similar approach using a permethylated allyl-substituted β -CD coupled to an organohydrosiloxane polymer. The preparation of a wall-coated capillary using an acrylamide polymer containing a β -CD derivative was described by Sze-man and Ganzler [218].

A comprehensive overview of the applications of cyclodextrins in chiral electrochromatography was recently published by Schurig and Wistuba [219]. Francotte and Jung [220] used neat cellulose derivatives, such as 3,5-dimethylphenylcarbamoyl cellulose and *p*-methylbenzoyl cellulose coated on silica gel and applied the same capillaries for CEC and OT-HPLC. A simple approach for preparation of chiral open tubular capillaries was reported by Liu *et al.* [221, 222]. Proteins, peptides and amino acids were adsorbed on the capillary wall by simply rinsing the capillary with a buffer containing the selector. These phases were evaluated using amino acids and some other compounds and showed remarkable results. Surprisingly, the lifetime of the capillaries was between one and several weeks.

Hofstetter *et al.* [223] reported the preparation of an open tubular capillary by immobilizing BSA *via* a silane to the capillary wall. The enantioselectivity was studied using DNP-amino acids and 3-hydroxy-1,4-benzodiazepines as model analytes. Hong *et al.* [224] described a similar approach using α_1 -acid glycoprotein (AGP), which was bound to the capillary wall by 3-glycidoxypropyltrimethoxysilane. Promethazine and benzocaine were used as test analytes. Regarding stability, the authors noted that one capillary showed only a small decrease in enantioselectivity after 50 days of use at room temperature. Sinibaldi *et al.* [205] coated capillaries with poly-terguride derived from ergot alkaloids and, on this, positively charged phase acidic analytes such as amino acid derivatives and flurbiprofen were resolved.

Molecularly imprinted polymers are used in CEC, either coated as a thin film to the capillary wall, or in the form of packed capillaries, or as monolithic phases. Several recent reviews [225–229] have been devoted to this basic technique. Generally, a chiral print molecule is loosely bound or entrapped in a polymer. After the print molecule is removed, a chiral imprint of this template remains, which is highly enantioselective for the same or very closely related molecules. Remcho and Tan [226] used L-Dns-phenylalanine as print molecule with methacrylic acid and 2-vinylpyridine as functional monomers and ethylene dimethacrylate as cross-linker in an *in situ* polymerization technique. To obtain a thin film at the capillary wall, the capillary was evacuated to effect shrinking of the polymer. The CSP showed high enantioselectivity for Dns-phenylalanine; efficiency, however, was rather low. Brügemann *et al.* [230] reported the preparation of very thin coatings. *S*(+)-2-phenylpropionic acid was used as a print molecule. Due to the extremely strong inclusion of the *S*(+) enantiomer only the *R*(-) enantiomer was detected; the *S*(+) enantiomer eluted as a very broad peak which was not detectable.

14.2 Packed capillaries

14.2.1 Achiral stationary phases with chiral mobile phases

Lelièvre *et al.* [231] used a reversed-phase capillary applying HP- β -CD as an additive to the mobile phase and compared the results with those obtained on a capillary packed with an HP- β -CD phase for the chiral separation of chlorthalidone and mianserin. Wei *et al.* [232] described the use of bare silica packing in combination with HP- β -CD as a mobile phase additive for the chiral separation of phenylephrine and synephrine. Deng *et al.* [233] resolved the enantiomers of salsolinol using a C18-packed capillary and β -CD as an additive to the mobile phase. 1-Heptane sulfonate was added as an ion-pairing reagent to improve the retention behavior of the analyte. Lämmerhofer and Lindner [234] resolved amino acid derivatives on an octadecyl silica (ODS) stationary phase using a quinine derivative as an ion-pairing reagent in the mobile phase.

14.2.2 Chiral stationary phase

Early investigations with packed capillaries deal with the use of β -CD [235] AGP- [236] and HSA- [237] HPLC-grade CSPs. Wistuba *et al.* [238] recently developed a packing material based on permethylated β -CD immobilized to 5 μm (mercaptopropyl) methyl-silica gel (Chirasil-Dex-silica 2) and evaluated this material by means of the chiral separation of barbiturates. The authors compared CEC, capillary HPLC and pressure-supported CEC using the same capillary in a CE system combined with a gradient pump. The authors have shown that with pressure-supported CEC, significantly shorter separation times and sharper peaks are obtained without significant loss of separation selectivity. Recently, Wistuba and Schurig [239] reported the preparation of a polysiloxane-linked permethyl- β -CD thermally immobilized on silica (Chirasil-Dex silica 1). The drawback of polymer-coated silica is that the EOF is reduced because the silanol groups are blocked; this can be overcome by adding unmodified bare silica [239]. In a recent review, Schurig and Wistuba [219] compare the results obtained with open tubular CD-coated capillaries and packed capillaries with CD-CSPs using both CEC and capillary LC.

Krause *et al.* [240] reported the use of poly-*N*-acryloyl-L-phenylalanine ethyl ester covalently bonded to silica (Chiraspher) or cellulose tris(3,5-dimethylphenylcarbamate) coated on silica for CEC, capillary HPLC and pressure-supported CEC and demonstrated the applicability of the different approaches for chiral separations of a broad spectrum of drugs. Recently, Krause *et al.* [180] synthesized a helically chiral poly(diphenyl-2-pyridylmethyl) methacrylate, which was coated to wide-pore

aminopropyl-silanized silica. These capillaries were used for capillary HPLC and nonaqueous CEC. The anodic EOF is assumed to be generated by not totally covered aminoalkyl groups of the aminopropyl-silanized silica gel.

Different groups have synthesized CEC-CSPs based on macrocyclic antibiotics [241–243]. Dermaux *et al.* [241] packed a capillary with a 5 μm vancomycin phase which is also used in HPLC and tested it with the chiral separation of warfarin and hexobarbital using triethylamine acetate (TEAA)/acetonitrile mixtures as mobile phase. Wikström *et al.* [242] prepared a new vancomycin CSP using a three-step *in situ* immobilization procedure on a packed diol-silica capillary. While vancomycin was used in CE for the chiral separation for acidic compounds, this vancomycin CSP could be applied to a broad spectrum of neutral, acidic, and basic compounds using a reversed-phase and polar organic mode. The resolution values for thalidomide in the reversed-phase mode were 2.5 (80 000 plates per meter) and in the polar organic mode 2.5 (120 000 plates per meter), respectively. Carter-Finch and Smith [243] packed a capillary with a teicoplanin CSP (Chirobiotic T 5 μm) and resolved on this phase tryptophan and dinitrobenzoyl leucine enantiomers using acetonitrile/phosphate buffer, pH 7, as mobile phase.

Wolf *et al.* [244] investigated two Pirkle-type CSPs. An (*S*)-naproxen-derived CSP and a (3*R*,4*S*)-Whelk-O-CSP based on 3 μm silica were packed into 100 μm ID capillaries. Excellent enantioselectivity was obtained on these CSPs for several test analytes with different structures using a MES (pH 6)/acetonitrile mixture as mobile phase with remarkable efficiency (up to 200 000 plates per meter). The chiral recognition mechanism is based on dipole-dipole stacking and π - π interactions. Lämmerhofer and Lindner [245] developed an anion exchange CSP based on *tert*-butyl quinine carbamate immobilized on 5 μm silica. This CSP was applied both for the HPLC and CEC separation of *N*-(9-fluorenylmethoxycarbonyl) amino acids and *N*-(3,5-dinitrobenzoyloxycarbonyl) amino acids. Ionic interactions and π - π interactions are assumed to be the main forces responsible for chiral recognition. Recently, the same group reported nonaqueous CEC using a CSP consisting of the same selector immobilized on 3 μm silica [181]. Acetonitrile/methanol mixtures containing acetic acid and triethylamine were used as mobile phases for the chiral resolution of amino acid derivatives. Compared to the previous published results, faster separations and improved efficiencies were obtained under these conditions. FMOC-Leu was separated in less than 10 min with a resolution factor of 6.9 and about 100 000 plates per meter.

Mayer *et al.* [246] and Francotte and Zhang [247] recently developed a new CSP based on a 3,5-dimethylphenylcar-

bamoyl cellulose phase immobilized on 5 or 7 μm silica gel of 4000 \AA pore size. Acetonitrile/phosphate or citrate buffers were used as mobile phases. This CSP was run both in the capillary LC and CEC mode. CEC showed much better column efficiency and enantioselectivity under similar conditions. Figure 6 shows typical electrochromatograms of some selected model analytes. An improvement in efficiency could certainly be obtained by using a silica gel of smaller particle size. Lin *et al.* [248] prepared molecularly imprinted polymers by copolymerization of methacrylic acid or 2-vinylpyridine as functional monomers and ethylene glycol dimethacrylate as cross-linker in the presence of 2,2'-azobis(isobutyronitrile) (AIBN) using Dns-L-leucine as print molecule. After sieving, the particles were packed into the capillary. The enantioselectivity was good for Dns-leucine but only low for other Dns-amino acids. In another paper these authors described the use of L-phenylalanine anilide as print molecule [249]. This CSP showed chiral recognition ability for phenylalanine and also slightly for other aromatic amino acids.

14.3 Monolithic CSPs

A recent trend in CEC is to move away from packed columns on a silica basis since reproducible packing of capillaries and the preparation of frits by sintering a packing zone requires some experience. Both the silica particles and the frits are sources of air bubbles. Air bubbles can be prevented by applying pressure at both ends of the capillary, but this requires special equipment. The prepara-

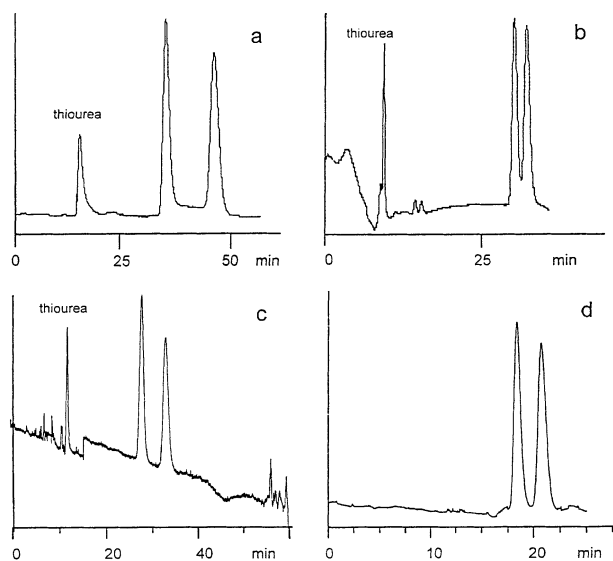


Figure 6. Electrochromatogram of the enantiomeric separation of (a) lormetazepam, (b) *trans*-stilbene oxide, (c) indapamide, (d) benzoin on a 3,5-dimethylphenylcarbamoyl cellulose phase. Reprinted from [246], with permission.

ration of monolithic phases on silica or polymer basis is a recent technique and certainly one of the most challenging approaches for the development of CSPs for CEC.

The technique of preparation of monolithic phases by *in situ* polymerization in the column was introduced by Hjertén *et al.* [250] in the 1980s already. To fix the polymer on the capillary wall, the capillary is first treated with γ -methacryloxypropyltrimethoxysilane, creating a double bond for copolymerization with the monomer solution. The monomer solution consists of a monomer, a cross-linker and a charge-forming agent. An allylated selector can be added. After degassing, the polymerization is initiated and the solution is drawn into the capillary. Intake must be stopped before the zone reaches the detection window. After 12 h the polymerization is complete.

Koide and Ueno [251] prepared monolithic CSPs by incorporating β -CD polymers such as poly β -CD and CM- β -CD in a polyacrylamide gel and separated terbutaline and benzoin enantiomers in these phases. Later, the same group [252, 253] prepared a positively charged monolithic CSP, to which allyl carbamoylated β -CD (AC- β -CD) was covalently bonded. A mixture of AC- β -CD, acrylamide, *N,N'*-methylenebisacrylamide and *N*-(2-acrylamidoethyl)-triethylammonium iodide in Tris / boric acid buffer containing *N,N,N',N'*-tetramethylethylenediamine and ammonium peroxydisulfate was filled into a capillary pretreated with γ -methacryloxypropyltrimethoxysilane. These monolithic CSPs showed good enantioselectivity for acidic compounds (*e.g.*, Dns-amino acids, warfarin, phenoxypropionic acid) and neutral compounds (benzoin and 1-(1-naphthalene) ethanol) with high efficiency (up to 150 000 plates per meter).

Peters *et al.* [254] prepared a "moulded" monolithic CSP by copolymerization of the chiral monomer 2-hydroxyethyl methacrylate (*N*-L-valine-3,5-dimethylanilide) carbamate with ethylene dimethylacrylate, 2-acrylamido-2-methyl-1-propanesulfonic acid and butyl or glycidyl methacrylate in the presence of a porogenic solvent. This phase has hydrophilic properties which can be enhanced further by hydrolysis of the glycidyl moieties. The chiral recognition ability of this monolithic CSP was demonstrated by means of *N*-(3,5-dinitrobenzoyl)leucine diallylamide. The column efficiency was found to be 61 000 plates per meter.

Recently, Schmid *et al.* [152] synthesized a monolithic ligand-exchange CSP using methacrylamide as a monomer, piperazine diacrylamide as a cross-linker, vinylsulfonic acid as a charge-providing agent and *N*-(2-hydroxy-3-allyloxypropyl)-L-4-hydroxyproline as a chiral selector. The polymer chains so yielded form a homogeneous network of interconnected nodules consisting of microparticles. The voids or channels between the nodules allow

high permeability and thus low back pressure. These chiral continuous beds are inexpensive and easy to prepare. This CSP was evaluated *via* a selection of underivatized amino acids. The same capillary was used for CEC, capillary LC and pressure-supported CEC (Fig. 7). Faster separations were obtained by “short-end injection”. In this case the effective length of the continuous bed was 8.5 cm and the enantiomers of phenylalanine were baseline-resolved within 4 min.

Schweitz *et al.* [255] prepared a monolithic imprinted polymer for chiral CEC separation by filling the capillary pretreated with γ -methacryloxypropyltrimethoxysilane with a prepolymerization mixture of print molecule, functional and cross-linking monomers (methacrylic acid and trimethylolpropane trimethacrylate), radical initiator AIBN and solvent (toluene) and polymerization was performed by placing the capillary under a UV source. The print molecule is removed by flushing the capillary with acetonitrile. An imprint remains, which shows high enantioselectivity for the same or very similar molecules. As print molecules (*R*)-propranolol [255, 256] or (*S*)-metoprolol [255] were used for chiral separation of β -blockers and (*S*)-ropivacaine for the resolution of local anesthetics. Lin *et al.* [257] developed a thermally induced *in situ* polymerization procedure for the preparation of an imprinted polymer using D-phenylalanine as a print molecule. Phenylalanine was baseline-resolved; tyrosine and phenylglycine showed only partial resolution.

15 ITP and IEF

Only a few publications deal with the application of ITP to chiral separations, although ITP was the first technique among electroseparation techniques applied for chiral separations [258–260]. The development of a powerful isotachophoretic sample pretreatment system coupled on-line to CZE was reported by Danková *et al.* [261]. ITP provides a preconcentration- and sample clean-up step enabling the interference-free determination of tryptophan enantiomers in a 90-component model mixture and urine. Figure 9 shows the electropherograms obtained with different working modes explicated in Fig. 8 using α -CD as chiral selector in the CZE system. The authors have clearly demonstrated that all interfering compounds were removed by transferring a narrow sample zone between two spacer constituents to the CZE system. With this approach, it was possible to detect 25 nmol/l L(-)Trp in the presence of the 200-fold amount of D(+)-Trp. Hoffman *et al.* [262] developed a continuous flow ITP system for the purification of *R*-(-)-methadone on an mg scale using HP- β -CD as a chiral selector [262]. Recently, Kaniansky *et al.* [263] published a new approach for the preparative separation of enantiomers by ITP. A column-coupling system was used consisting of a pre-separation column of

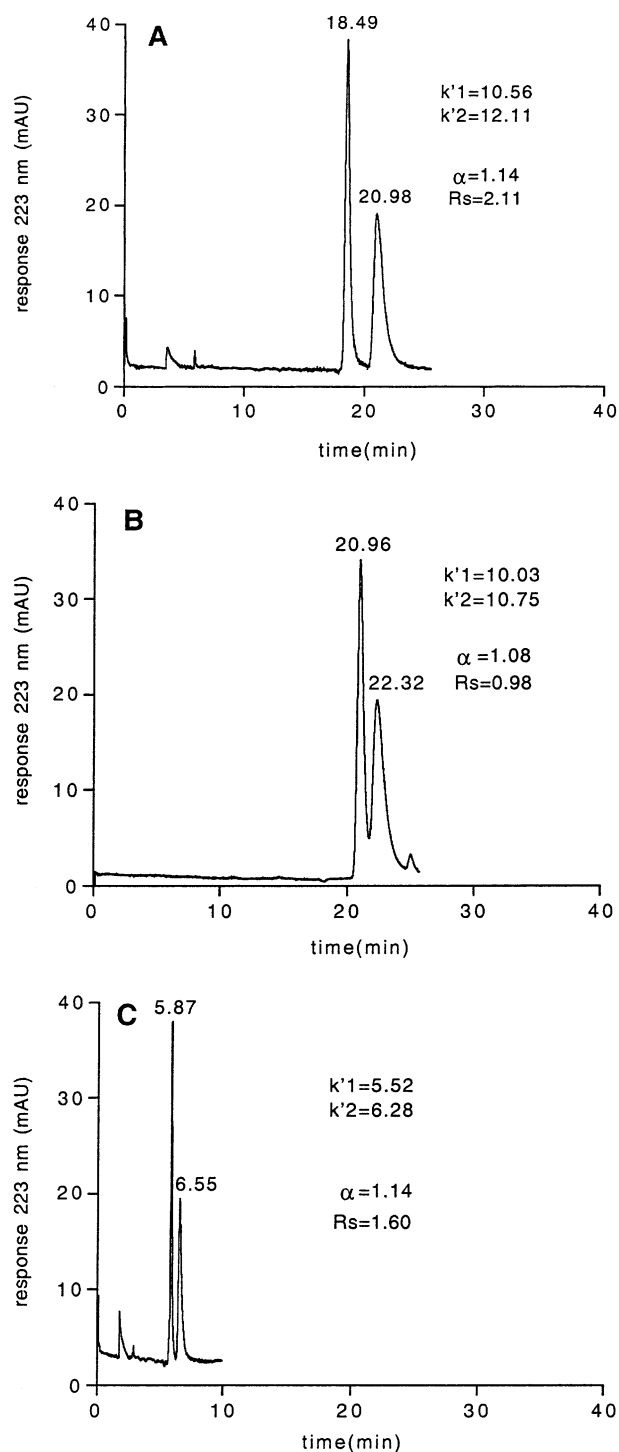


Figure 7. Chiral separation of Phe comparing (A) CEC, (B) pressure-driven micro-HPLC and (C) pressure-supported CEC on a monolithic LE-CSP. Conditions: stationary phase, monolithic ligand-exchange CSP (26 cm \times 75 μ m); mobile phase, 50 mM sodium dihydrogen phosphate / 0.1 mM Cu(II), pH 4.6; injection, 10 kV \times 6 s; (A) 30 kV, (B) 12 bar, (C) 30 kV and 12 bar. Reprinted from [152], with permission.

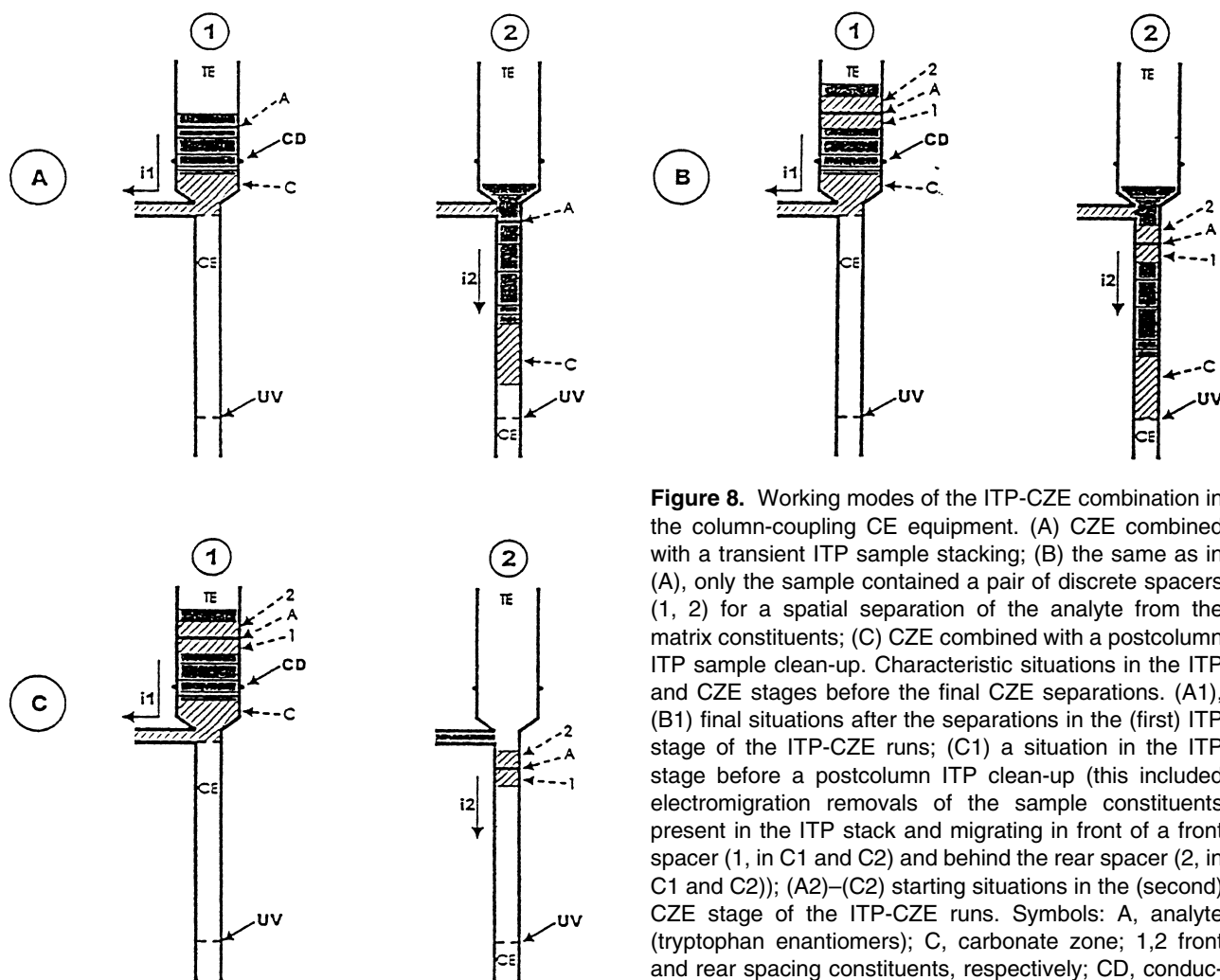


Figure 8. Working modes of the ITP-CZE combination in the column-coupling CE equipment. (A) CZE combined with a transient ITP sample stacking; (B) the same as in (A), only the sample contained a pair of discrete spacers (1, 2) for a spatial separation of the analyte from the matrix constituents; (C) CZE combined with a postcolumn ITP sample clean-up. Characteristic situations in the ITP and CZE stages before the final CZE separations. (A1), (B1) final situations after the separations in the (first) ITP stage of the ITP-CZE runs; (C1) a situation in the ITP stage before a postcolumn ITP clean-up (this included electromigration removals of the sample constituents present in the ITP stack and migrating in front of a front spacer (1, in C1 and C2) and behind the rear spacer (2, in C1 and C2)); (A2)–(C2) starting situations in the (second) CZE stage of the ITP-CZE runs. Symbols: A, analyte (tryptophan enantiomers); C, carbonate zone; 1,2 front and rear spacing constituents, respectively; CD, conductivity detector; UV, photometric absorbance detector. Reprinted from [261], with permission.

1.0 mm ID and a trapping column of 0.8 mm ID. β -CD was used as chiral selector separating DNP-leucine enantiomers as a model analyte. The authors have shown that up to 14 mg of pure DNP-norleucine enantiomers could be obtained in one preparative run. A recent paper deals with the use of transient ITP-CZE for chiral separations [264]. A counterflow is generated by applying counterpressure at the capillary. The system was applied to the separation of clenbuterol enantiomers using dimethyl- β -cyclodextrin as chiral selector. Preparative IEF was used by Gluckhovsky and Vigh [265] for the separation of Dns-phenylalanine enantiomers on mg/h scale.

16 Indirect separation

Little work has been done in this field in recent years. Thorsén *et al.* [266] described the synthesis of a new chiral precolumn derivatization reagent for amino compounds, (+) and (–) 1-(9-anthryl)-2-propyl chloroformate.

This reagent was applied to the chiral separation of 17 amino acids and some small peptides by MEKC using SDS in borax buffer in combination with LIF detection. Kleidernigg and Lindner [267] synthesized (1*R*,2*R*)- and (1*S*,2*S*)-*N*-[(2-isothiocyanato)cyclohexyl]-6-methoxy-4-quinolinyamide) as chiral derivatization reagent for amino acids. The diastereomeric derivatives were separated by RP-HPLC and quasi-CEC using PVP as a pseudostationary phase.

Another chiral derivatization reagent for amino acids and peptides, *R*-(-) or *S*-(+) 4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole was described by Liu *et al.* [268]. The resulting derivatives were separated by micelle-mediated CE using a nonionic surfactant Triton X-100 in acetate buffer, pH 4. The fluorescent derivatives were detected by LIF detection using an argon-ion laser. An exciting application of a new derivatization procedure with the goal of determining amino acids and their enan-

tiomeric ratio in micrometeorites was recently reported by Trambouze-Vandenabeele *et al.* [269]. The amino acids

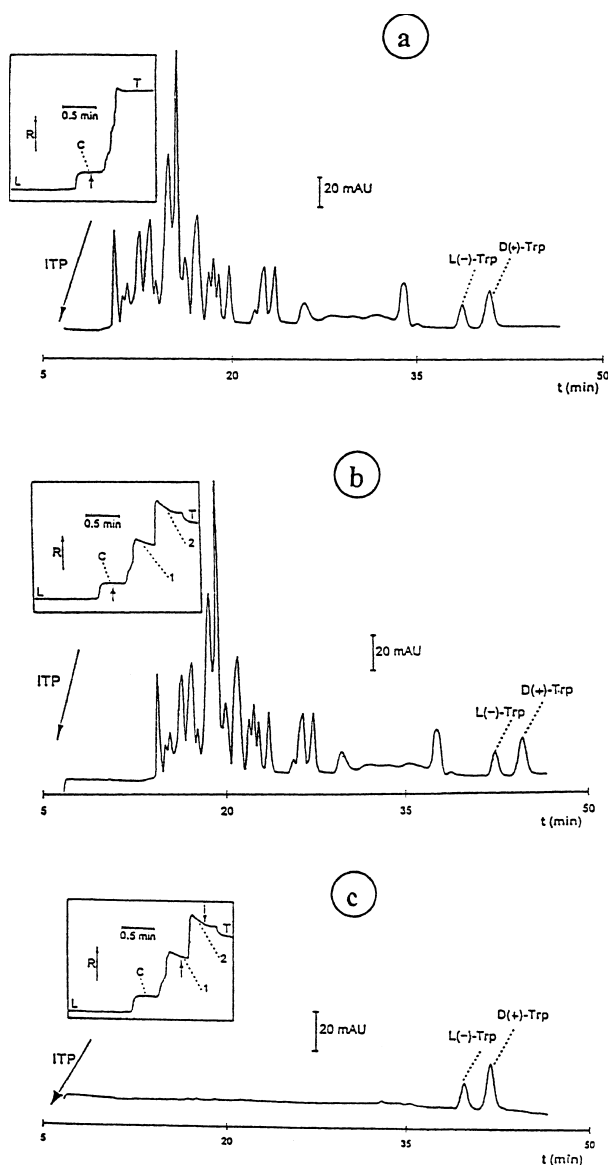


Figure 9. Electropherograms from the separations of tryptophan enantiomers present in a 90 component model mixture of organic acids using various working modes of the ITP-CZE combination. (a)–(c) Electropherograms corresponding to the working modes (A)–(C), respectively, shown in Fig. 8. L(–)-Trp and D(+)-Trp were present in the injected mixture (30 μ L) at 0.5 μ mol/L and 0.8 μ mol/L concentrations, respectively. The concentrations of organic acids in the model mixture were in the range of 1–5 μ mol/L. Arrows on the isotachopherograms mark the sample fraction taken for final CZE separations. Glycine (1) and β -alanine (2) added to the sample at 1 mmol/L concentrations served as discrete spacing constituents in the ITP stage of the separation. Reprinted from [261], with permission.

were derivatized using a chloroethylnitrosourea of ϵ -benzoyloxycarbonyl-lysine-*t*-butyl ester and FITC. The separation of the diastereomeric derivatives was carried out with borate buffer in the presence of the nonionic surfactant Brij-58 using LIF. The low detection limits (about 10 pM for isovaline) make the method suitable to detect trace amounts of amino acid enantiomers in micrometeorites.

17 Miscellaneous

Reversal of the EMO is often of importance, especially with enantiomeric purity check of drugs. The enantiomer present as an impurity in a sample of the active enantiomer should always appear as the first peak to avoid being covered by the tailing of the major component. Chankvetadze *et al.* [270] recently published an excellent survey of various possibilities of reversing the EMO and discussed the mechanisms of the different approaches. The simplest possibility is to use selectors possessing opposite chirality. This is not possible, however, with natural selectors. It was found that different CDs and CD-derivatives can exhibit different chiral recognition ability for the enantiomers. The EMO can be reversed by changing from a neutral to a charged CD. Also, oligosaccharides of different type or chain length were found to respond differently to the enantiomers. Changing the mobility of the analyte by varying the pH is another way of reversing the EMO. On the other hand, the same effect can be obtained by changing the mobility of the chiral selector or by changing the relation of the mobilities of the analyte and the selector and reversing the polarity. Suppressing or reversing the EOF are further means for reversing EMO. Simply the change from an uncoated to a coated capillary may reverse the EMO. EOF can be reversed, for example, by using hydrophobic quaternary ammonium compounds, which form a positively charged layer at the capillary wall. In some cases, EMO was even seen to depend on selector concentration. The combination of different chiral selectors is another tool for reversing the EMO and last but not least, charged micelle forming achiral surfactants are frequently added to reverse EMO.

A new technique, synchronous cyclic CE, was recently introduced by Zhao and Jorgenson [271]. The high resolving power was demonstrated by the application of this technique to isotopic and chiral separations. Over 100 million plates are reported to be reached with this system. Chiral separation of (α -hydroxybenzyl)methyltrimethylammonium and (2-hydroxy-1-phenyl)ethyltrimethylammonium, compounds which could not be separated with any other system, was achieved with this technique using β -CD as chiral selector. The combination of flow injection (FI) with CE for the chiral separation of intermediate enantiomers in chloramphenicol synthesis was described

by Liu and Fang [272]. CD derivatives were used as chiral selectors. The samples were injected continuously by FI without current interruption, achieving a five times higher sample throughput with improved precision compared to electrokinetic injection.

Palmarsdottir *et al.* [273] described the development of a supported liquid membrane technique using a microcolumn LC interface for on-line coupling with a CZE system for sample pretreatment and preconcentration. In this system, bambuterol in subnanomole concentrations was detected in human plasma. Using permanently charged CDs, Vigh's group [37] developed the CHARM model, which is based on the consideration of simultaneous protonation and complexation equilibria. This model is helpful for selecting the appropriate operating conditions based on rational predictions. Another approach to give assured predictions for optimal separation is the combination of artificial neural networks of experimental design to optimize the separation of amino acid enantiomers using α -CD as a chiral selector [274].

18 Microchips

Two years ago, the feasibility of using microfabricated lab-on-a-chip systems for the chiral separation of labeled amino acids was demonstrated. Hutt *et al.* [275] resolved FITC-labeled amino acids on a microfabricated CE chip to show how such devices can analyze for extinct or extant signs of life in extraterrestrial environments. The test system consisted of a folded electrophoresis channel (19.0 cm long \times 150 μ m wide \times 20 μ m deep) that was photolithographically fabricated in a 10 cm diameter glass wafer sandwich; laser-excited confocal fluorescence was used for detection, providing subattomole sensitivity. They used an SDS / γ -CD, pH 10.0, carbonate electrophoresis buffer and a separation voltage of 550 V/cm at 10°C. In only 4 min, baseline resolution was observed for Val, Ala, Glu, and Asp enantiomers. Another approach was published by Rodriguez *et al.* [276] again using CD-modified MEKC for the chiral separation of FITC-amino acids on a microfabricated device. In their experiments, analysis time ranged from 75 s for basic amino acids to 160 s for acidic amino acids. Efficiency up to 395 000 plates/m was reached, promising that the use of such devices could lead to a challenging new field.

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