ELSEVIER

Journal of Chromatography A, 875 (2000) 255–276

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Review

Enantiomer separation of chiral pharmaceuticals by capillary electrochromatography

Dorothee Wistuba*, Volker Schurig

Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle 18, D-72076 Tübingen, Germany

Abstract

Enantiomer separation of chiral pharmaceuticals by capillary electrochromatography (CEC) is achieved with open-tubular capillaries (o-CEC), with packed capillaries (p-CEC) or with monolithic capillaries. In o-CEC, capillaries are coated with a thin film containing cyclodextrin derivatives, cellulose, proteins, poly-terguride or molecularly imprinted polymers as chiral selectors. In p-CEC, typical chiral HPLC stationary phases such as silica-bonded cyclodextrin or cellulose derivatives, proteins, glycoproteins, macrocyclic antibiotics, quinine-derived and 'Pirkle' selectors, polyacrylamides and molecularly imprinted polymers are used as chiral selectors. Chiral monolithic stationary phases prepared by in situ polymerization into the capillary were also developed for electrochromatographic enantiomer separation. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Enantiomer separation; Electrochromatography; Pharmaceutical analysis; Molecular imprinting; Chiral selectors; Saccharides; Proteins; Peptides; Glycoproteins; Antibiotics

Contents

1. Introduction 256
2. Capillary electrochromatographic methods and application to enantiomer separation 256
 2.1. Open-tubular capillary electrochromatography (o-CEC) 256
  2.1.1. Saccharides (cyclodextrins, cellulose) 256
  2.1.2. Proteins (bovine serum albumin, lysozyme, cytochrome c) and peptides 261
  2.1.3. Anion-exchange type stationary phase 261
  2.1.4. Molecularly imprinted polymers 261
 2.2. Packed capillary electrochromatography 262
  2.2.1. Saccharides (cyclodextrins, cellulose derivatives) 263
  2.2.1.1. Chiral selector covalently attached to silica 263
  2.2.1.2. Chiral selector added to the mobile phase 265
  2.2.2. Proteins and glycoproteins 267
  2.2.3. 'Pirkle' phase 268
  2.2.4. Macrocyclic antibiotics (vancomycin, teicoplanin) 268
  2.2.5. Anion-exchange type stationary phases 269
  2.2.6. Polyacrylamide derivatives 272

*Correspondence author: Tel.: +49-7071-2978-762; fax: +49-7071-295-538.
E-mail address: dorothee.wistuba@uni-tuebingen.de (D. Wistuba)

0021-9673/00/$ – see front matter © 2000 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(00)00066-2

2.2.7. Molecularly imprinted polymers ... 272
2.3. Electrochromatography with monolithic chiral stationary phases ... 272
3. Comparison of enantiomer separation by o-CEC with p-CEC and monolithic CEC ... 273
4. Conclusion and future prospects ... 274
5. Abbreviations ... 275
Acknowledgements ... 275
References ... 275

1. Introduction

Capillary electrochromatography (CEC) [1–3] represents a hybrid method of capillary electrophoresis (CE) and liquid chromatography (LC). Whereas in chromatography differences in partition coefficient between two phases is responsible for separation, the separation mechanism in CE is based on differences in the rate of migration in the electric field. If both the electrophoretic and the chromatographic features of separation are combined, the method is called CEC. Thus CEC combines the high efficiency of capillary electromigration with the selectivity of chromatography.

Enantiomer separation by CEC [4,5] has received considerable attention in recent years and has evolved as a powerful technique in the analysis of chiral pharmaceuticals. Enantioselective drug analysis requires a highly efficient and sensitive method because trace enantiomer analysis and the analysis of very complex mixtures are often necessary. Enantioselective CEC can be divided into three main modes:

(i) Open-tubular electrochromatography (abbreviated o-CEC, OT-EC or OT-CEC), in which the internal capillary wall is coated with the chiral stationary phase (CSP). Such columns are also used in GC, SFC or o-LC.
(ii) Packed CEC, in which capillaries are filled with typical chiral HPLC packing materials (p-CEC, packed CEC, micro-packed CEC or μ-CEC).
(iii) Continuous rod electrochromatography, in which capillaries with monolithic chiral stationary phases prepared by in situ polymerization methods within the capillaries are employed.

2. Capillary electrochromatographic methods and application to enantiomer separation

2.1. Open-tubular capillary electrochromatography (o-CEC)

In o-CEC the chiral selector is coated and immobilized onto the inner surface of a capillary column and the different distribution equilibria between the mobile phase and the coated chiral stationary phase (CSP) are responsible for the enantiomer separation of a racemic selectant. Such wall-coated open-tubular capillaries are commonly used in gas chromatography (GC), supercritical fluid chromatography (SFC) and also in open-tubular liquid chromatography (o-LC). In fact, a unified enantioselective approach can be realized by employing a single column in all chromatographic modes [6–8,13].

The first instance of enantiomer separation by o-CEC was described by Mayer and Schurig in 1992 with capillaries coated with immobilized Chirasil-Dex [9–12]. Apart from saccharides (cyclodextrins, cellulose) [5,9–16], proteins [bovine serum albumin (BSA), lysozyme, cytochrome *c*] [17–20] and peptides [19,20] have been investigated as chiral selectors in o-CEC. Imprinting polymers were also designed as chiral stationary phase in o-CEC [21–23].

2.1.1. Saccharides (cyclodextrins, cellulose)

Chirasil-Dex, a permethyl-β-cyclodextrin covalently linked via an octamethylene spacer to a dimethylpolysiloxane (see Fig. 1), was used for enantiomer separation of a number of non-steroidal anti-inflammatory drugs and barbiturates [9–12]. Chirasil-Dex was thermally immobilized resulting in

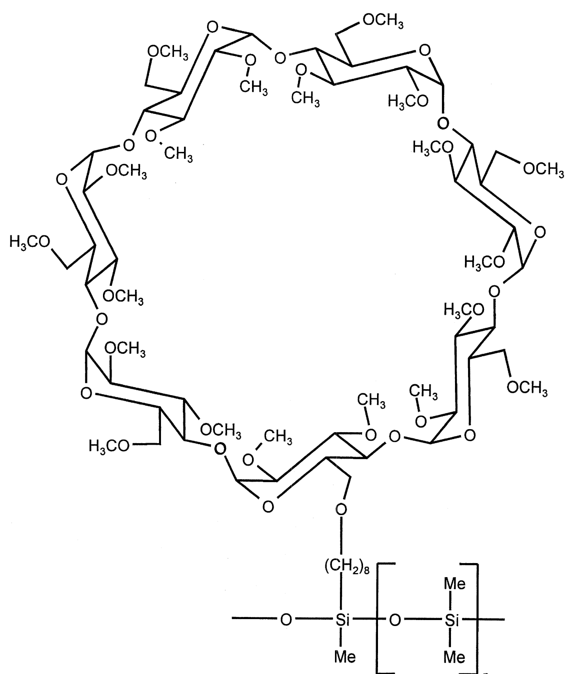


Fig. 1. Structure of Chirasil-Dex (dependent upon the reaction conditions, the carrier may be linked to the 2- or 6-position of cyclodextrin).

non-extractable and buffer-resistant wall-coated open-tubular columns, which were stable under neutral and acidic (pH 2.5) conditions for several months [11]. Fig. 2 shows the enantiomer separation of the underivatized non-steroidal anti-inflammatory drugs flurbiprofen, cicloprofen and ibuprofen using an 85-cm×50-μm I.D. Chirasil-Dex coated column [10,11]. Chirasil-Dex modified capillaries could also be used for enantiomer separation of barbiturates such as hexobarbital or mephobarbital (see Fig. 3 and Table 1) [7,8,11,24]. While etodolac shows no separation on Chirasil-β-Dex coated columns, baseline resolution was achieved by employing Chirasil-γ-Dex [11]. Mephobarbital, 5-(2-propyl)-5-(*n*-propyl) barbituric acid, and 5-ethyl-5-(*n*-propyl) barbituric acid were also separated on capillaries coated with Chirasil-DiMe-β-Dex (dimethylated β-cyclodextrin covalently linked to dimethylpolysiloxane) [25].

The EOF in untreated capillaries depends strongly on the negative charges of the silanol groups of the internal wall surface. The coating of the capillary with Chirasil-Dex leads to a decrease of the EOF (about 30% in comparison to an untreated column), because the free silanol groups on the capillary surface responsible for the EOF are masked in the course of the thermal crosslinking process or even partially removed by chemical reaction with the polydimethylsiloxane matrix [11]. Hence, migration time are rather long. The film thickness strongly influences the electrochromatographic parameters such as plate number N and resolution R_s. The best results were obtained with a film thickness of 0.2 μm. While with increasing film thickness the plate number decreases, resulting in peak broadening, a very thin film (0.1 μm) leads to loss of sample capacity [9].

The addition of an organic modifier to the background electrolyte changes migration times and selectivities. The influence of various amounts of methanol, 2-propanol and acetonitrile on the enantiomer separation of mephobarbital was investigated. While the number of theoretical plates N increased with increasing amounts of all investigated organic modifiers, the resolution R_s was reduced with higher concentration of modifier [8].

Recently, Schurig et al. [6–8] reported on the principle of open-tubular *unified enantioselective chromatography* using a single column coated with Chirasil-Dex for all important contemporary methods of enantiomer separation, i.e., GC, SFC, o-LC and o-CEC. Fig. 4 shows the feasibility of the unified enantioselective approach for the enantiomer separation of hexobarbital on a 1-m×50-μm I.D. capillary coated with Chirasil-Dex. Apart from the long migration time, o-CEC is superior to GC, SFC and o-LC in respect to: (i) chiral separation factor α (o-CEC≈o-LC>SFC>GC); (ii) peak resolution R_s (o-CEC>SFC≈GC>o-LC); (iii) efficiency N (first peak) (o-CEC>o-LC>GC>SFC).

A related approach was demonstrated by Armstrong et al. [13] for the enantiomer separation of mephobarbital on a wall-immobilized permethylated β-cyclodextrin covalently linked to dimethylpolysiloxane via trimethylene spacers.

Addition of another chiral selector to the running buffer during an enantiomer separation on a chiral stationary phase leads to the concept of *dual chiral recognition* [12,24]. Mayer et al. [12] and Jakubetz et al. [24] studied the enantiomer separation of

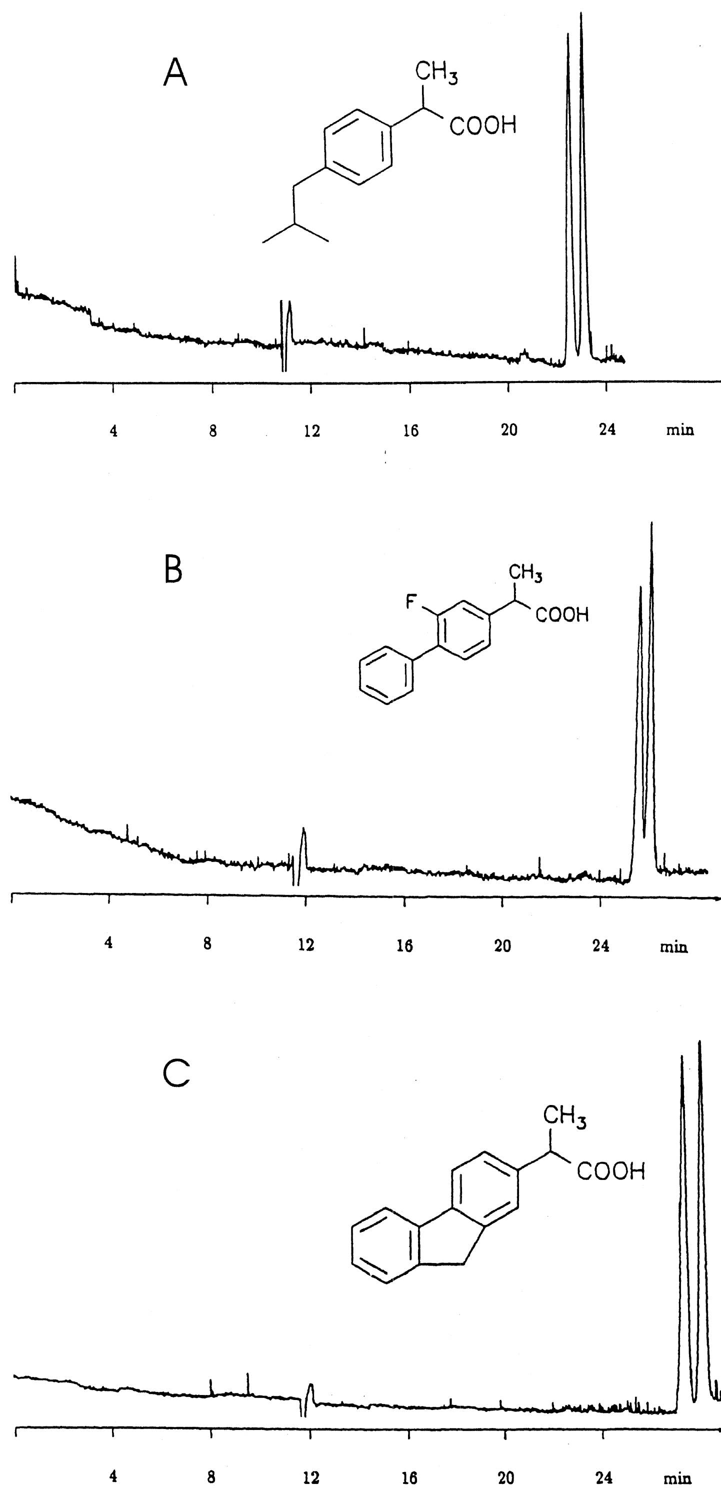


Fig. 2. Enantiomer separation of ibuprofen (A), flurbiprofen (B) and cicloprofen (C) by o-CEC; 85 cm×50 μm I.D. capillary coated with immobilized Chirasil-Dex. Conditions: Tris–HCl (pH 7), 30 kV [10].

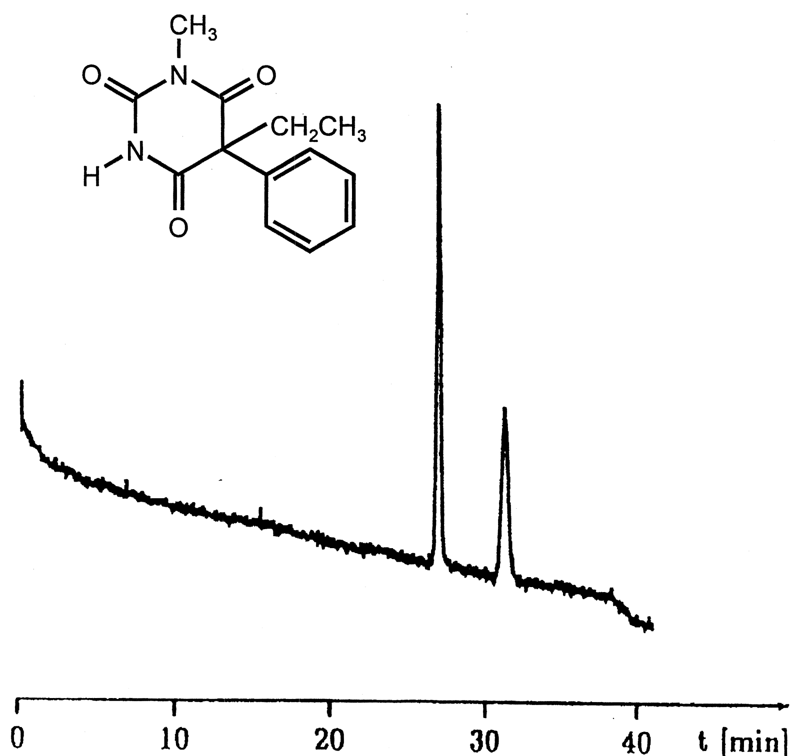


Fig. 3. Enantiomer separation of mephobarbital by o-CEC; 90 cm×50 μm I.D. capillary coated with immobilized Chirasil-Dex. Conditions: borate–phosphate buffer (20 m*M*, pH 7), 30 kV [8].

hexobarbital at pH 7 in a combined system employing a Chirasil-Dex coated capillary in combination with either the anionic SPE-β-cyclodextrin (6-*O*-(sulfo-*n*-propyl)-β-cyclodextrin) or the cationic HTAP-β-cyclodextrin (β-cyclodextrin-2-hydroxy-3-trimethylammoniumpropylether chloride) as running buffer additive. Hexobarbital can be well separated by Chirasil-Dex (migration order, *R* before *S*), by addition of anionic SPE-β-cyclodextrin to the mobile phase of an untreated capillary (*S* before *R*) or by addition of cationic HTAP-β-cyclodextrin to the mobile phase of a polyacrylamide-coated column (*R* before *S*). As expected, the addition of HTPA-β-cyclodextrin to the mobile phase of a Chirasil-Dex coated capillary leads to an increase in the chiral separation factor α, while a decrease of α was observed upon addition of the negatively charged SPE-β-cyclodextrin (see Fig. 5).

Table 1
Enantiomer separation of chiral pharmaceuticals on Chirasil-β-Dex [9–12]

Substrate	N/m[a]	R_s
Ibuprofen	30 700	1.97
Flurbiprofen	34 900	1.64
Cicloprofen	18 600	2.32
Carprofen	1400	0.95
Warfarin	113 900	1.20
Hexobarbital	9170	8.32
Mephobarbital	18 800	6.00
5-(2-Propyl-5-(*n*-propyl) barbituric acid	1600	2.40
5-Ethyl-(1-methylbutyl) barbituric acid	1700	1.30

[a] Second eluted enantiomer.

Another method for immobilization of β- and γ-cyclodextrins was developed by Sezemán and Ganzler [14]. Either an acrylamide layer is bound to the capillary wall prior to the cyclodextrin coating, or cyclodextrins are linked to the double bonds present in the linear acrylamide by catalysis with Ce(IV) salts. With a γ-cyclodextrin-coated capillary the enantiomers of epinephrine were separated. Un-

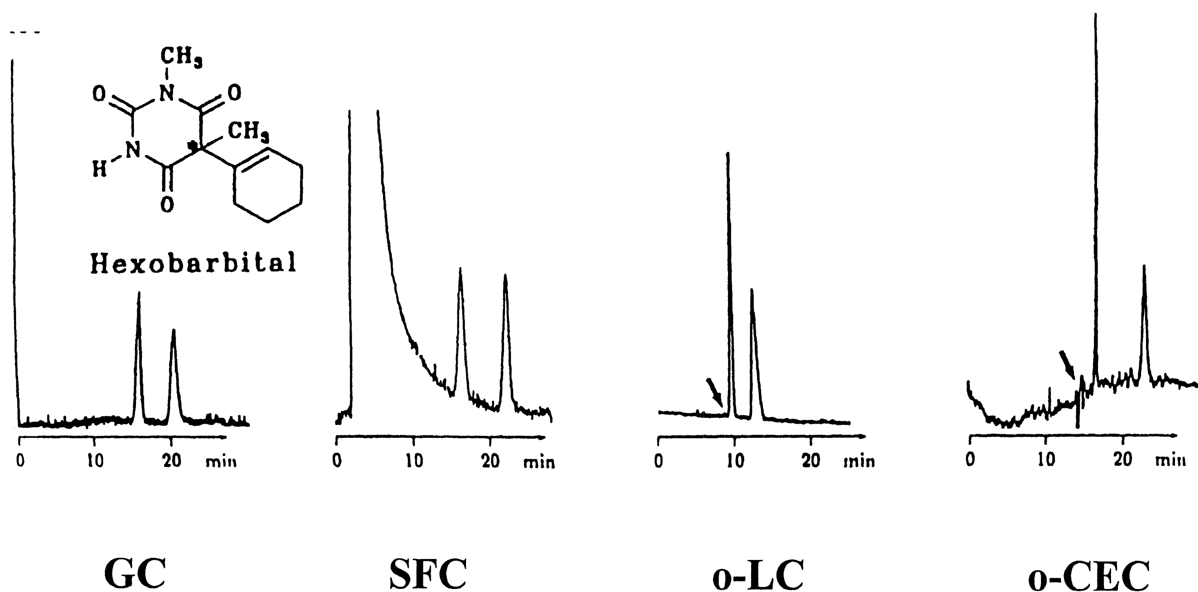


Fig. 4. Enantiomer separation of hexobarbital on a 1 m×50 μm I.D. capillary coated with immobilized Chirasil-Dex by GC, SFC, o-LC and o-CEC. Effective column length in o-LC and o-CEC, 85 cm. GC, 1 bar He, 155°C; SFC, 0.31 g/ml CO_2, 60°C; o-LC, 0.2 bar, [borate–phosphate buffer (pH 7)]–methanol (80:20, v/v), 20°C; o-CEC, 30 kV, borate–phosphate buffer (pH 7), 20°C [7].

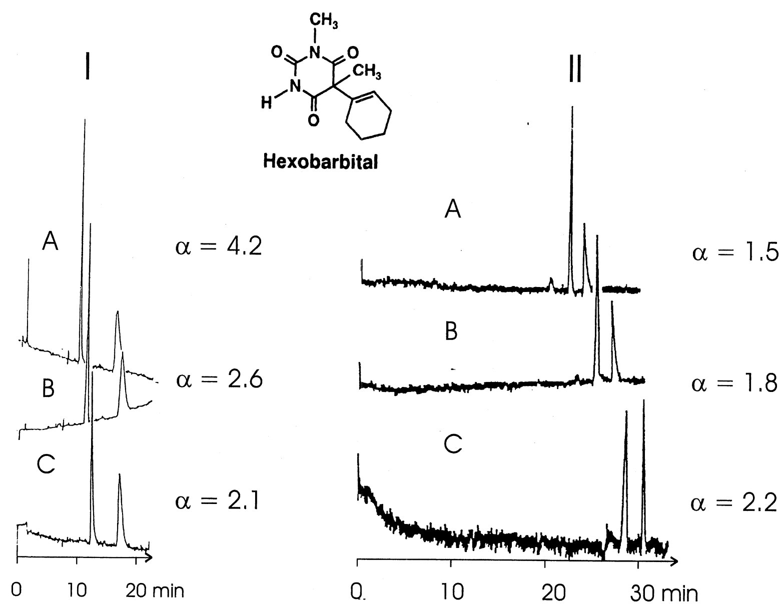


Fig. 5. Enantiomer separation of hexobarbital in the dual chiral recognition mode: (I) 80 cm×50 μm I.D. capillary coated with immobilized Chirasil-Dex; 30 kV; 20 m*M* borate–phosphate buffer (pH 7). (A) Without chiral additive, (B) 0.5 m*M* SPE-β-CD, (C) 1 m*M* SPE-β-CD. (II) 80 cm×50 μm I.D. capillary coated with Chirasil-Dex; 30 kV; [borate–phosphate buffer (pH 7)]–acetonitrile (90:10, v/v). (A) Without chiral additive, (B) 1 m*M* HTAP-β-CD, (C) 5 m*M* HTAP-β-CD [24].

fortunately, the cyclodextrin coating proved to be stable at neutral pH only for up to 50 injections.

Pesek et al. [15] attached 2-hydroxyl-3-methacryloyloxypropyl-β-cyclodextrin to the inner wall of etched capillaries. Partial resolution of the enantiomers of several benzodiazepines was achieved. With chiral lactone or naphthylamine instead of cyclodextrin, partial separation of the tricyclic depressants, doxepine, nortriptyline and clomipramine and derivatized amino acids was feasible [15].

The unique enantioselective properties of cellulose derivatives in HPLC have been transferred into the o-CEC system by Francotte and Jung [16]. Enantiomer separation of mephobarbital, glutethimide and aminoglutethimide was performed on a non-immobilized stationary phase consisting of neat 3,5-dimethylphenylcarbamoyl cellulose or 4-methylbenzoyl cellulose. Disadvantages of this method are the short lifetime of the column (only 100 injections) and an unstable EOF.

2.1.2. Proteins (bovine serum albumin, lysozyme, cytochrome c) and peptides

Direct covalent binding of bovine serum albumin (BSA) to the internal surface of a capillary results in a stable stationary phase suitable for enantiomer separation in o-CEC [17]. These capillaries are operable up to 1 year when stored properly at 4°C. On-column UV detection was possible in the whole range of the detection wavelength without limitation caused by protein absorption. Enantiomer separation of oxazepam, lorazepam and several DNP-amino acids has been performed with high resolution. The drugs oxazepam and lorazepam elute in the form of typical interconversion profiles with the appearance of a plateau between the peaks of the enantiomers caused by rapid enantiomerization of 3-hydroxy-1,4-benzodiazepines (see Fig. 6).

Usually the adsorption of basic proteins and peptides on the capillary wall is undesired in CE and may lead to serious problems. Liu and co-workers [18–20] used an adsorbed protein or peptide layer as stationary phase for enantiomer separation in o-CEC. Lysozyme and cytochrome c with very high isoelectric points (11.1 and 10.7, respectively), are suitable as chiral stationary phases. The drug mephenytoin and several derivatized amino acids were separated

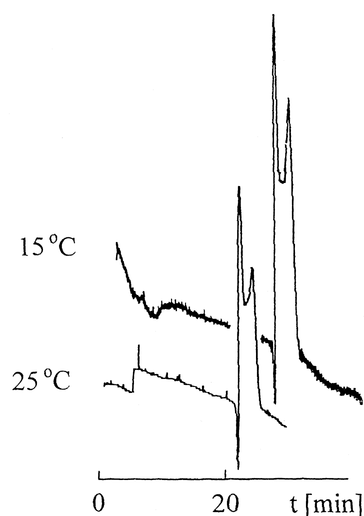


Fig. 6. Enantiomer separation of oxazepam by o-CEC displaying enantiomerization; 60 cm×50 μm I.D. capillary derivatized with BSA. Conditions: phosphate buffer (50 m*M*, pH 8.0), 7.5 kV [17].

into enantiomers with a resolution from 1.74 to 2.05. Basic peptides such as Lys–Tyr and Lys–Ser–Tyr have been applied as stationary phases for enantiomer separation of tyrosine.

2.1.3. Anion-exchange type stationary phase

Capillaries coated with poly-terguride [26], an alkaloid-based chiral stationary phase, were used for enantiomer separation of the negatively charged fluprofen and derivatized amino acids. In the pH range between 2.5 and 4.0 an anodic electroosmotic flow was observed which is caused by the positively charged moiety of the ergolinic skeleton. Migration of the enantiomers was affected by the concentration and the composition of the mobile phase.

2.1.4. Molecularly imprinted polymers

Crosslinked polymers possessing chiral cavities were prepared by mixing functional monomers in presence of enantiomerically pure print molecules. After polymerization the covalently or non-covalently bonded print molecule was removed. Resulting polymers contain structure-specific recognition sites with affinity for the original chiral imprint molecule [23]. Molecularly imprinted polymers (MIPs) have been employed in the electrochromatographic enantiomer separation of a number of chiral drugs

[21,22]. An advantage of MIP is that the choice of a suitable chiral selector is not based on the *trial and error* concept and the elution order is predictable. The imprint enantiomer is the more strongly retained and it is therefore eluted last. In o-CEC, thin films of MIPs are bonded to the internal wall of a capillary. Tan and Remcho [22] used an in situ polymerization technique and methacrylic acid and 2-vinylpyridine were chosen as functional monomers, ethylene dimethacrylate or trimethylol propane trimethacrylate as crosslinker and L-dansyl-phenylalanine as print molecule. With such coated capillaries (25 μm I.D.) the enantiomer separation of D- and L-dansyl-phenylalanine was realized. The efficiency of the non-imprinted D-dansyl phenylalanine was high (248 600 theoretical plates/m), but the imprinted L-dansyl phenylalanine eluted as a broad peak with low efficiency (8000 theoretical plates/m) (see Fig. 7). Brüggemann et al. [21] described a method for preparing MIP films with thicknesses of below 0.1 μm in capillaries with 100 μm I.D. However, no direct enantiomer separation was possible due to the very strong interaction of the print molecule (one enantiomer), which elutes as a very broad peak and thus cannot be distinguished from the baseline noise.

2.2. Packed capillary electrochromatography

In packed capillary electrochromatography (p-CEC), enantiomer separation is performed in a capillary filled with a typical HPLC packing material [4,5]. The packing bed is held in position by frits (sintered silica) and detection occurs in the empty part of the column. In comparison to p-LC, p-CEC offers higher efficiency leading to narrow peaks with increasing detectability. Moreover, coupling to MS-systems is more straightforward. CEC offers also the possibility of decreasing the particle size of the

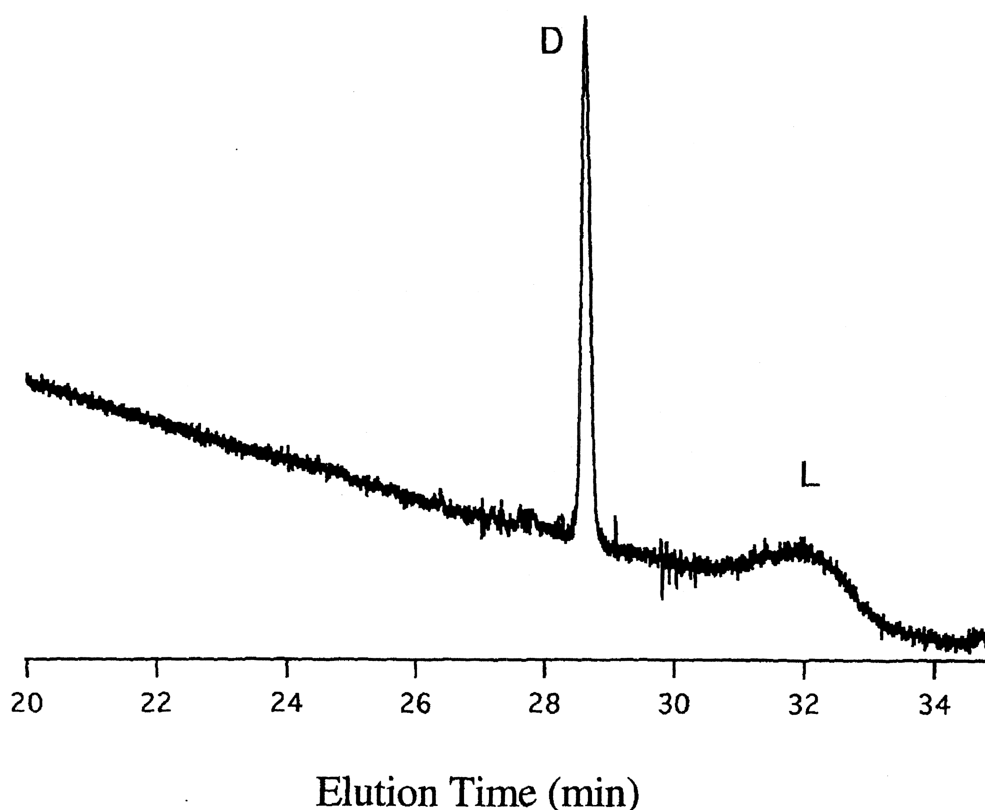


Fig. 7. Enantiomer separation of a mixture of D- and L-dansyl phenylalanine by o-CEC on a MIP [22] coated capillary (85 cm×25 μm I.D.). Conditions: acetonitrile–phosphate buffer (10 m*M*, pH 7) (10:1, v/v), 30 kV. Reprinted with permission from Ref. [22].

packing material without a large increase in pressure drop. A serious problem in p-CEC is the tendency toward bubble formation inside the column. This effect usually leads to an instability or even a breakdown of the current and can be circumvented by pressurizing the system. p-CEC can be realized in two main modes:

(i) the chiral selector is covalently bound to the silica particles which were filled into a column; and
(ii) the chiral selector is added to the running buffer of a capillary packed with silica or modified silica.

2.2.1. Saccharides (cyclodextrins, cellulose derivatives)

2.2.1.1. Chiral selector covalently attached to silica

Cyclodextrin or modified cyclodextrin-immobilized stationary phases were employed for enantiomer separation of a number of chiral drugs in p-CEC [5] (see Table 2). A native β-cyclodextrin stationary phase was used by Li and Lloyd [27] for the enantiomer separation of neutral racemates such as hexobarbital and anionic racemates such as derivatized amino acids. As compared to the phosphate buffer, the employment of triethylammonium acetate (TEAA) as background electrolyte reversed the direction of the EOF by adsorption of cations onto the silica surface. The reversal of the EOF is desirable for the analysis of anions. Enantioselectivity is only observed in water or aqueous organic solutes. Methanol, ethanol and acetonitrile were used as organic modifier with the best selectivity found for methanol. Increasing the amount of methanol leads only to slight changes in migration time because the effect of reduction in retention is partially compensated by the reduction of the EOF. The efficiency of the packed capillary system is similar to an open-tubular system using β-cyclodextrin as additive with a TEAA–methanol mobile phase. The stability of β-cyclodextrin-linked silica is pH dependent (pH 3.5–7.5) and primarily determined by that of the silica. Lelièvre et al. [28] reported on the enantiomer separation of the neutral drug chlorthalidone possessing diuretic and hypertensive activity and the antidepressive cationic drug mianserin on a capillary packed with hydroxypropyl-β-cyclodextrin-linked silica. While the enantiomers of chlorthalidone was baseline separated, only a slight separation was observed for mianserin. The observed broad tailing peak is a result of the electrostatic interaction of the cationic mianserin and the negatively charged silica particles. The influence of acetonitrile as organic modifier were studied in the range between 15 and 80%. Increase of the acetonitrile content leads to a decrease in the chiral separation factor α and the resolution R_s but to an increase in the migration time t.

Permethylated β-cyclodextrin [5] as chiral selector in p-CEC was successfully employed using two approaches:

(i) the permethylated β-cyclodextrin was covalently linked via a thioether spacer (see Fig. 8) to silica (Chira-Dex–silica) [29]; and
(ii) the permethylated β-cyclodextrin was linked to dimethylpolysiloxane and thermally immobilized on silica (polymer-coated Chirasil-Dex–silica) [30] (see Fig. 1).

Barbituric acids (see Fig. 9), glutethimide, benzoin, α-methyl-α-phenylsuccinimide were separated by p-CEC on both permethylated Chira-Dex–silica and polymer-coated Chirasil-Dex–silica. To avoid bubble formation during analysis the flow system was pressurized by coupling an HPLC pump to the injector buffer vial. All runs were performed with a pressure of 10–15 bar. This technique, called pressure-supported p-CEC, offers the advantage of switching between p-LC and CEC mode in a single instrumental set-up, of flushing the column and of simple operation in the gradient mode.

Fig. 10 demonstrates the influence of the pressure support on the enantiomer separation of mephobarbital on Chira-Dex–silica [29]. The elution time is clearly dominated by a substantial contribution of the EOF. Comparing p-LC at 10 bar with p-CEC at 10 bar and 20 kV, the elution time found in p-LC are longer by a factor of approximately 10 for Chira-Dex–silica [29] and of approximately three for Chirasil-Dex–silica [30] at comparable chiral separation factors and resolutions. The theoretical plate numbers N are generally higher in the CEC mode than in the p-LC mode. Fig. 11 shows the comparison of the separation of mephobarbital by p-CEC and p-LC on Chira-Dex–silica [29]. At comparable migration times, efficiency and resolution are clearly

Table 2
Enantiomer separation of chiral pharmaceuticals by p-CEC on cyclodextrin-derived stationary phases

Substrate	Mobile phase	R_s	Refs.
Hexobarbital	4 m*M* Phosphate, 15% methanol, pH 6.8	1.39	[27]
	5 m*M* TEAA, 15% acetonitrile, pH 4.71	1.50	[27]
	5 m*M* Phosphate, pH 7.0, 20% methanol	2.56	[29]
	20 m*M* MES, pH 6.0, 50% methanol	3.81	[30]
Mephobarbital	5 m*M* Phosphate, pH 7.0, 20% methanol	3.00	[29]
	20 m*M* MES, pH 6.0, 50% methanol	4.34	[30]
Pentobarbital	5 m*M* Phosphate, pH 7.0, 20% methanol	0.89	[29]
1-Methyl-5-(2-propyl)-5-(*n*-propyl)barbituric acid	5 m*M* Phosphate, pH 7.0, 20% methanol	0.85	[29]
	20 m*M* MES, pH 6.0, 60% methanol	2.15	[30]
5-Ethyl-1-methyl-5-(*n*-propyl)barbituric acid	5 m*M* Phosphate, pH 7.0, 20% methanol	2.3	[29]
	20 m*M* MES, pH 6.0, 50% methanol	3.05	[30]
Chlortalidone	5 m*M* Phosphate, pH 6.5, 25% acetonitrile	1.7	[28]
Benzoin	4 m*M* Phosphate, 15% methanol, pH 6.8	0.85	[27]
	5 m*M* Phosphate, pH 7.0, 20% methanol	1.37	[29]
	20 m*M* MES, pH 6.0, 50% methanol	1.36	[30]
Glutethimide	5 m*M* Phosphate, pH 7.0, 20% methanol	1.35	[29]
	20 m*M* MES, pH 6.0, 60% methanol	1.84	[30]
α-Methyl-α-phenyl-succinimide	5 m*M* Phosphate, pH 7.0, 20% methanol	1.01	[29]
	20 m*M* MES, pH 6.0, 50% methanol	1.19	[30]

higher in the CEC mode with both Chira-Dex–silica- and Chirasil-Dex–silica-packed columns. The chiral separation factor remained nearly unchanged. The influence of the organic modifier methanol and acetonitrile on migration time, resolution and efficiency on the enantiomer separation of mephobarbital was studied. The best enantioselectivity was observed with methanol as organic modifier. With increasing content of organic modifier, the theoretical plate number increases while the resolution and the chiral separation factor decreases. For the elution of various substances on polymer-coated Chirasil-

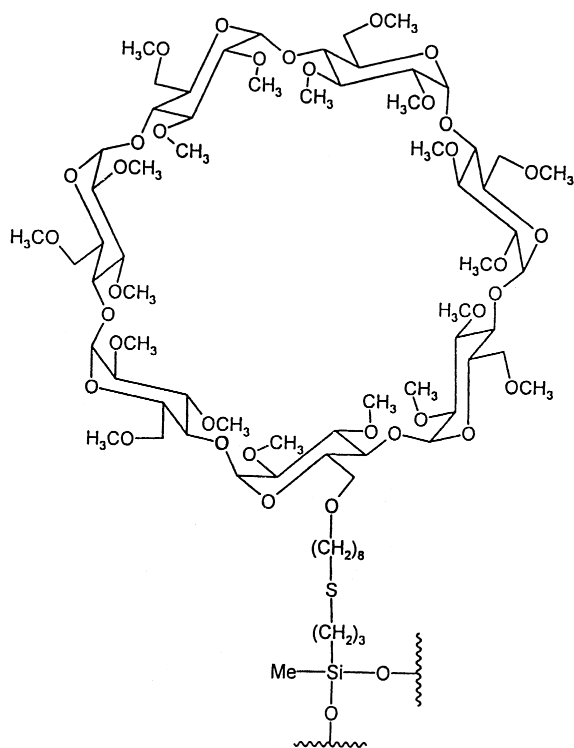


Fig. 8. Structure of Chira-Dex–silica (dependent upon the reaction conditions, the carrier may be linked to the 2- or 6-position of cyclodextrin).

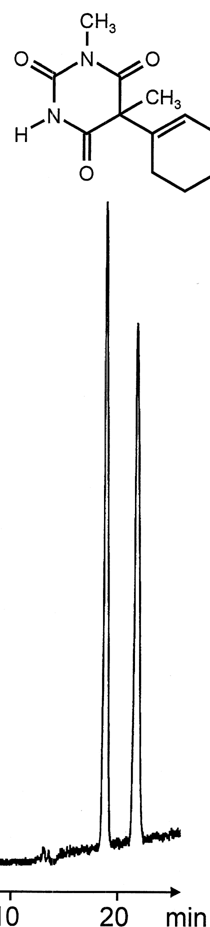


Fig. 9. Enantiomer separation of hexobarbital by p-CEC on a 25 cm×100 μm I.D. capillary filled with a mixture of 80% Chirasil-Dex–silica and 20% bare silica. Conditions: MES buffer (20 m*M*, pH 6.0)–methanol (1:1, v/v), 20 kV, 10 bar; UV detection, 230 nm [30].

Dex–silica, a higher amount of the modifier methanol is required than with Chira-Dex–silica [30]. Polymer-coating is, in principle, detrimental to CEC since the EOF is reduced by shielding part of the free silanol population by thermal crosslinking and by chemical reaction with residual Si–H groups in the dimethylpolysiloxane matrix remaining after synthesis of Chirasil-Dex. This effect is easily overcome by adding unmodified bare silica to the packing material [30]. For comparable results a higher amount of the modifier methanol is necessary to remove the analytes from the apolar dimethylpolysiloxane. In selected cases the enantioselectivity of polymer-coated Chirasil-Dex–silica differs from that of Chira-Dex–silica, e.g., the enantiomers of 1-(2-naphthyl)ethanol were only resolved on Chirasil-Dex–silica [30].

Polysaccharide derivatives are also suitable as chiral selector in p-CEC. Recently, Krause et al. reported on the enantiomer separation of indapamide on cellulose tris(3,5-dimethylphenylcarbamate) coated on silica by pressure-supported CEC [31] (see Fig. 12).

2.2.1.2. Chiral selector added to the mobile phase

The common practice to add a chiral selector into the mobile phase can also be adopted in p-CEC. Thus, for enantiomer separation a capillary was packed either with bare silica or ODS (octa-

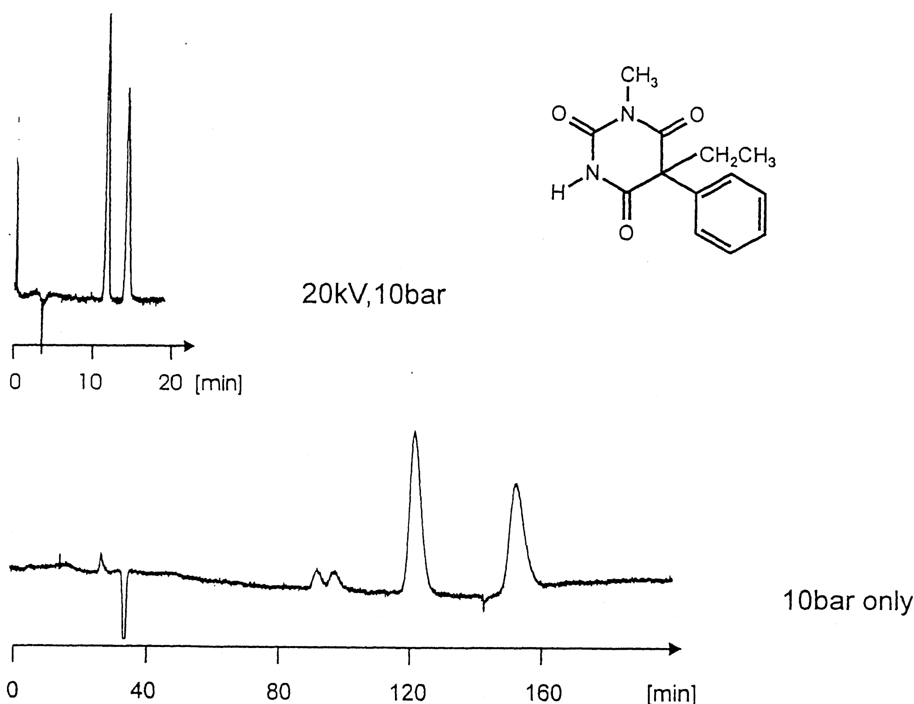


Fig. 10. Influence of pressure support on the enantiomer separation of mephobarbital by p-CEC. Column: 23.5 cm×100 μm I.D. capillary packed with Chira-Dex–silica. Conditions: phosphate buffer (5 m*M*, pH 7.0)–methanol (4:1, v/v); UV detection, 230 nm; upper electropherogram, 20 kV, 10 bar; lower electropherogram, 10 bar [29].

decylsilica) and cyclodextrin derivatives were added as chiral selectors to the electrolyte.

Lelièvre et al. [28] added hydroxypropyl-β-cyclodextrin to the mobile phase of an ODS packed column. Chlorthalidone enantiomers were separated with resolution factors between 0.7 and 1.4 depending on the content of the organic modifier acetonitrile. Three main factors responsible for enantiomer separation were discussed:

(i) differences in the stability constants of inclusion complexes between hydroxypropyl-β-cyclodextrin and the enantiomers;

(ii) differences in partition of the inclusion complexes between the two phases; and

(iii) differences in partition of the free enantiomers between the mobile phase and the hydroxypropyl-β-cyclodextrin layer arising from adsorption on the surface.

Enantiomer separation takes place only after an equilibration time of 15 h. Compared with enantiomer separations of chlorthalidone on a hydroxypropyl-β-cyclodextrin–silica-packed capillary (see Section 2.2.1.1), longer migration times and lower resolution factors were found (see Section 2.2.1.1). Deng et al. [32] developed a theoretical model with regard to enantioselectivity and resolution for CEC and compared it with the experimental data obtained for the enantiomer separation of the endogenous neurotoxin salsolinol using an ODS-packed column and β-cyclodextrin as mobile phase additive. The theoretical model shows that the advantage of the combination of electrophoretic and partitioning mechanisms in CEC is the increase in enantioselectivity. For pressure-supported CEC, whereby the solvent is mainly driven by pressurized flow, the increased enantioselectivity was significantly reduced.

Recently, Wei et al. [33] demonstrated the feasibility of enantiomer separation by CEC on capillaries packed with bare silica using hydroxypropyl-β-cyclodextrin as mobile phase additive. They supposed that hydroxypropyl-β-cyclodex-

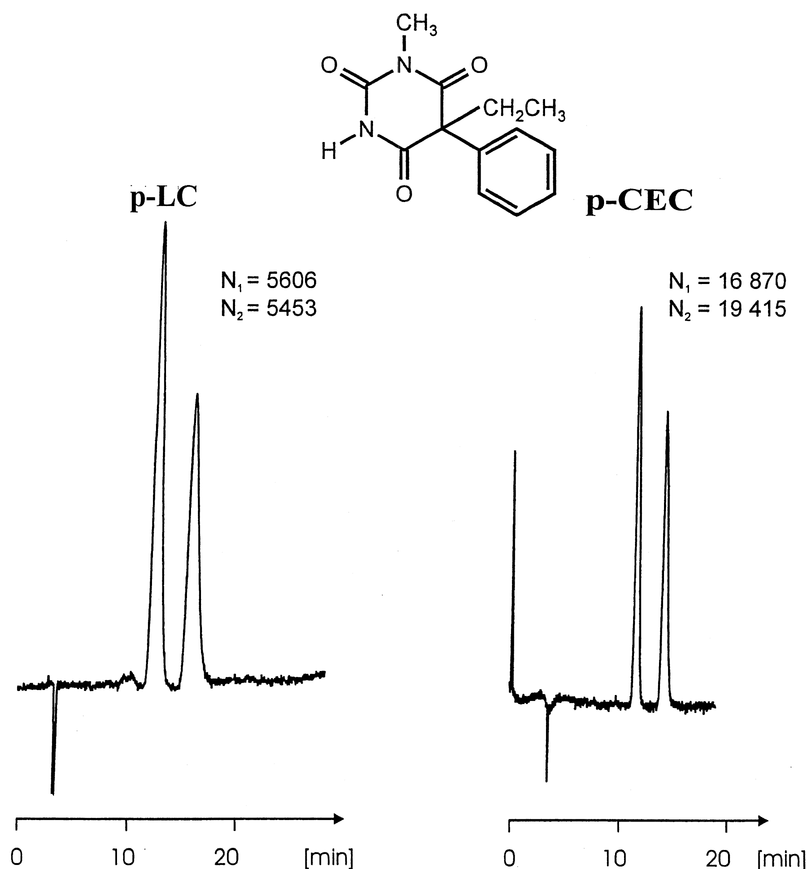


Fig. 11. Enantiomer separation of mephobarbital by p-LC and p-CEC. Column: 23.5 cm×100 μm I.D. capillary packed with Chira-Dex–silica. Conditions: phosphate buffer (5 m*M*, pH 7.0)–methanol (4:1, v/v); UV detection, 230 nm; CEC, 20 kV, 10 bar; μ-HPLC, 140 bar [29].

trin is adsorbed on silica and may assist enantiomer separation. Enantiomers of synephrine and phenylephrine were separated by CEC and by CZE, both with hydroxypropyl-β-cyclodextrin as chiral additive. The resolution is lower and the migration time is about three times longer in CZE than in CEC. The pH influences strongly the migration time, showing a cation exchange mechanism, but only slightly the resolution. The addition of methanol leads to a decrease in migration times and resolutions and to an increase in theoretical plate numbers.

2.2.2. Proteins and glycoproteins

It is well known that proteins adsorb easily on the internal capillary wall and may therefore cause serious problems such as peak tailing, loss of recovery, instability of the baseline and changes in the migration time in CZE. Another disadvantage is the strong detector response. To overcome these problems, p-CEC with proteins immobilized on silica can be used. Li and Lloyd [34] packed capillaries of 50 μm I.D. with an α_1-acid glycoprotein (AGP) chiral stationary phase and achieved an enantiomer separation of benzoin, hexobarbital, pentobarbital, ifosfamide, cyclophosphamide, disopyramide, metoprolol, oxprenolol and propranolol. Unfortunately, often broad and tailed peaks were observed, although efficiency was in general higher than that found in HPLC using the AGP phase. However, efficiency was much lower than that observed in CZE. AGP has an isoelectric point of 2.7 and over the normal operating pH range both capillary wall and immobil-

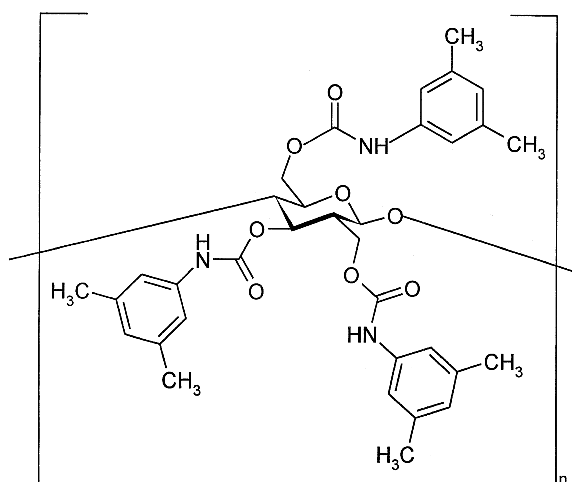
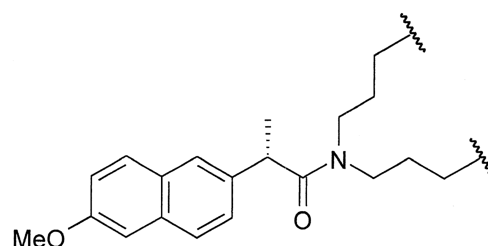


Fig. 12. Structure of cellulose tris(3,5-dimethylcarbamate).

ized protein are negatively charged. Enantiomer separation was performed using methanol, ethanol, 1-propanol, 2-propanol and acetonitrile as organic modifiers with the best resolution being obtained with 2-propanol. Retention factor and enantioselectivity decrease with increasing amount of organic modifier. An increase of the pH of the electrolyte leads to a decrease of the migration time without noticeable influence on the enantioselectivity. Electrochromatographic enantiomer separation has also been performed with a human serum albumin (HSA) as the stationary phase [35]. Enantiomer separation of benzoin and temazepam with various amounts of organic modifiers such as 1- and 2-propanol and acetonitrile has been described. The best enantioselectivity was found with 2-propanol or acetonitrile. Efficiencies of 15 000 plates/m for benzoin and 7000 plates/m for temazepam are very low and comparable with those obtained in HPLC. For both protein stationary phases, AGP and HSA, the efficiency is much lower than that observed for CEC with cyclodextrin stationary phases. Better results should be expected if smaller particles than those described (AGP, 5 μm; HSA, 7 μm) were to be used.

2.2.3. *'Pirkle' phase*

Wolf et al. [36] immobilized naproxen-derived and Whelk-O-type stationary phases (see Fig. 13) on silica (3 μm) and found high efficiencies (up to 200 000 plates/m) and excellent resolution factors



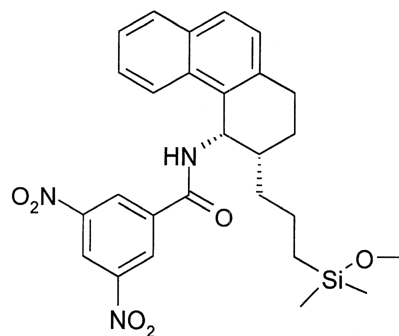


Fig. 13. Structure of *S*-naproxen-derived CSP and 3*R*,4*S*-Whelk-O CSP.

(between 2.6 and 31) combined with short migration times (less than 10 min) for electrochromatographic enantiomer separation of some neutral substances. The best results were obtained using 2-(*N*-morpholino)ethanesulfonic acid (MES) modified with acetonitrile as mobile phase. Bubble formation in the capillary was prevented by pressurizing the system on the inlet and outlet buffer vials (10 bar). A typical electropherogram is shown in Fig. 14.

2.2.4. *Macrocyclic antibiotics (vancomycin, teicoplanin)*

The macrocyclic antibiotics vancomycin and teicoplanin, widely used as chiral selectors in HPLC, have also been employed in p-CEC [37–39]. Capillaries packed with vancomycin-coated silica were used for enantiomer separation of warfarin and hexobarbital with efficiencies of up to 39 000 plates/m [37]. Comparing with results obtained by HPLC, 30% higher efficiency values were observed in the

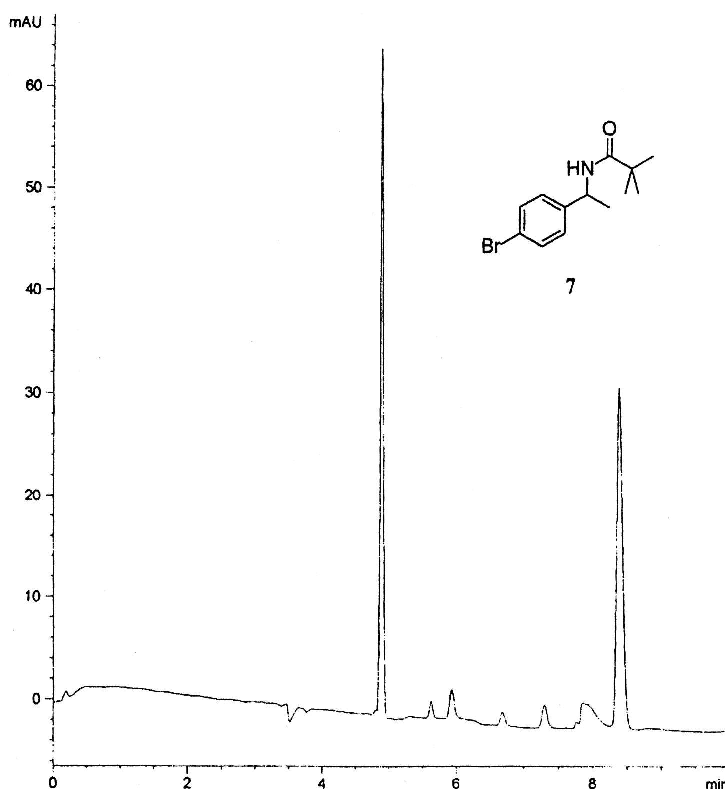


Fig. 14. Enantiomer separation of *N*-[1-(4-bromo-phenyl)ethyl]-2,2-dimethyl-propionamide on 3*R*,4*S*-Whelk-O CSP by p-CEC. Conditions: MES buffer (25 m*M*, pH 6.0)–acetonitrile (1:3.5, v/v); 25 kV; UV detection, 230 n*M*. Column, 30.5 cm×100 μm I.D. Reprinted with permission from Ref. [36].

CEC mode. Acetonitrile was suitable as an organic modifier for enantiomer separation, but with tetrahydrofuran no enantiomer separation was observed. Recently, thalidomide enantiomers were separated in the normal-phase and in the reversed-phase mode on a vancomycin chiral stationary phase [38]. Carter-Finch and Smith [39] reported on the enantiomer separation of the antidepressant drug tryptophan (see Fig. 15) and a derivatized amino acid (dinitrobenzoyl leucine) using a packed capillary with teicoplanin (see Fig. 16) covalently bonded to silica as chiral stationary phase. Methanol, ethanol and acetonitrile were studied as organic modifiers. The best enantioselectivity was observed for ethanol. But compared with acetonitrile, ethanol leads to longer migration time.

2.2.5. Anion-exchange type stationary phases

Lämmerhofer and Lindner [41,42] reported on packed-capillary electrochromatography using WAX-type stationary phases based on chiral quinine carbamate selectors (see Fig. 17). The quinine-derived selector was either bonded to silica [41] or added to the mobile phase using an ODS-packed capillary [42]. With both methods a number of derivatized amino acid enantiomers were separated (see Fig. 18). The quinine selector provides two basic amino groups: the tertiary quinuclidine and the aromatic quinoline group with different p*K* values. In the typically working range (pH 5.5–6) the quinuclidine nitrogen group is fully protonated and carries a positive charge (ion-paring with the anionic analytes), while the quinoline group is preferentially non-ionized. At pH values above 6.2, a cathodic flow and below 6.2, an anodic flow was observed using the stationary phase with covalently bonded selector [41]. The efficiency of the system operating in the p-CEC mode, measured on the weakly interacting

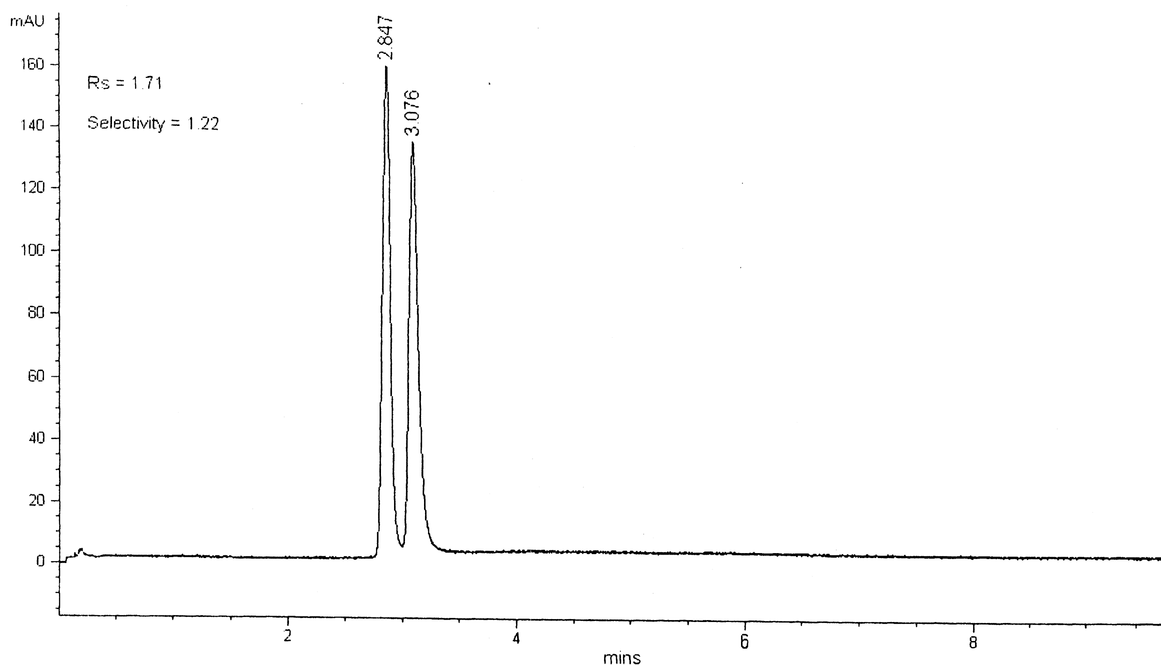


Fig. 15. Enantiomer separation of tryptophan by p-CEC on a 24.5 cm×100 μm I.D. capillary filled with teicoplanin bonded to silica. Conditions: Na_2HPO_2 (2 m*M*, pH 7.0)–acetonitrile (3:7, v/v); 30 kV; UV detection, 214 nm. Reprinted with permission from Ref. [39].

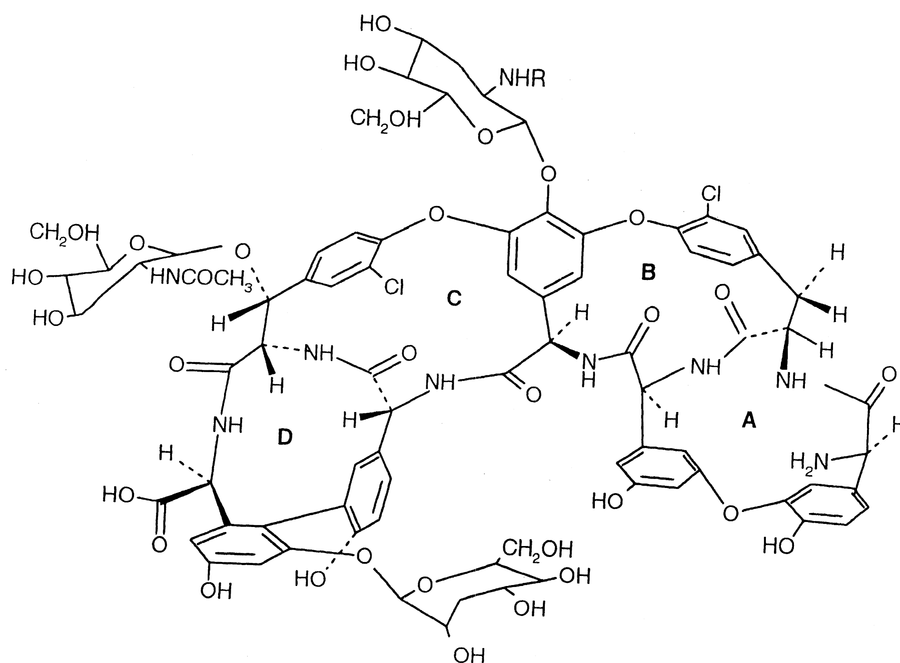


Fig. 16. Structure of teicoplanin. Reprinted with permission from Ref. [39].

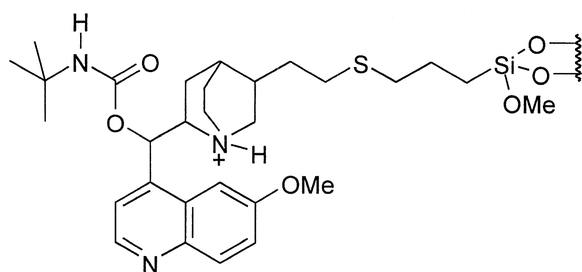


Fig. 17. Structure of a chiral quinine carbamate selector.

enantiomer, is approximately twice that obtained in the HPLC mode while the enantioselectivity remained unchanged. For the first eluted enantiomer, about 122 000 plates/m have been obtained. Only 42 000 plates/m were found for the second eluted enantiomer (HPLC: 60 700 plates/m). Typical organic modifiers used were methanol and acetonitrile. The buffer concentration influences the migration time (increasing buffer concentration causes a decrease of the migration time) but essentially not the enantioselectivity. Using ODS-packed capillaries in combination with quinine-derived selector as mobile phase additive [42], the enantioselectivity, the migration time and the elution order can be controlled by two different modes.

(i) Electrophoretically dominated mode: a high background electrolyte concentration leads to high efficiency (two to three times higher than in LC) but to only moderate enantioselectivity (about the same as in LC). The electrophoretic mobility of the anionic solutes is higher than its

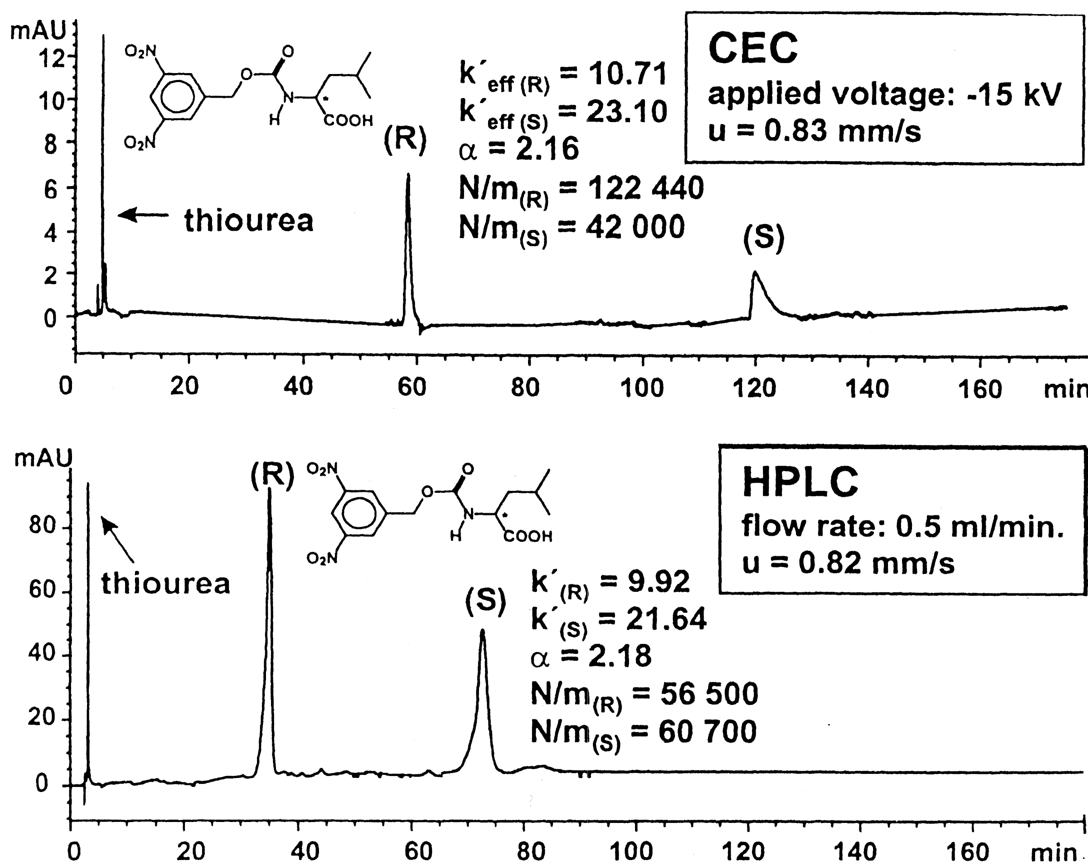


Fig. 18. Enantiomer separation of *N*-(3,5-dinitrobenzyloxycarbonyl)-leucine on quinine carbamate-based WAX-type CSPs by p-CEC. Column: 25 cm×100 μm I.D. Conditions: acetic acid (50 m*M*)–acetonitrile (1:4, v/v) (mixture titrated to pH 6.0 with triethylamine); UV detection, 254 nm.; CEC, −15 kV, 8 bar (inlet and outlet); HPLC, flow-rate, 0.5 ml/min. Reprinted with permission from Ref. [41].

electroosmotic mobility and thus dominates the separation. Detection occurs with negative electric polarity.

(ii) Electroosmotically dominated mode: at low electrolyte concentration, the chiral ion-pair agent (quinine-derived selector) itself serves as background electrolyte exclusively and the electrophoretic mobility of the anionic solutes is overcompensated by the cathodic eluent mobility. Detection occurs with positive electric polarity.

In this case a higher enantioselectivity, but much lower efficiency (even inferior to LC) was observed. The enantiomer separations carried out with the two modes (i) and (ii) resulted in inversion of the order of elution. It is possible to work in a non-aqueous media (methanol), which allows higher concentrations of the chiral ion-pair agent.

2.2.6. Polyacrylamide derivatives

The electrochromatographic enantiomer separation of bendroflumethiazide was demonstrated on a capillary packed with silica modified by covalent attachment of poly-*N*-acryloyl-L-phenylalanineethyl ester (Chiraspher) [31] (see Fig. 19), a well-known chiral selector in HPLC. To avoid bubble formation the inlet vial was pressurized to 12 bar, the outlet vial to 4 bar. Acetonitrile as organic modifier leads to better results than methanol.

Helically chiral poly(diphenyl-2-pyridylmethyl-methacrylate) coated on wide-pore aminopropyl silica was used for the enantiomer separation of Tröger's base, benzoin acetate, methylbenzoin and *trans*-stilbene oxide in nonaqueous pressure supported CEC [40].

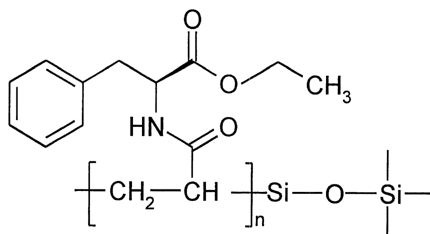


Fig. 19. Structure of poly-*N*-acryloyl-L-phenylalanine ethyl ester.

2.2.7. Molecularly imprinted polymers

The preparation of MIPs are described in Section 2.1.4. For p-CEC the polymers obtained were ground and sieved to particles <10 μm and then packed in capillaries [43–45]. Enantiomer separation of several derivatized amino acids were described. Using dansyl-L-leucine as print molecule, methacrylic acid and/or 2-vinylpyridine as functional monomers, ethylene glycol dimethacrylate as cross-linking monomer and AIBN as reaction initiator, only the resolution factor ($R_s=1.20$) of dansyl-D,L-leucine was satisfactory [43]. When the network was prepared using L-phenylalanine anilide or L-phenylalanine as print molecule the enantiomer separation of a number of aromatic amino acids was possible [44]. Comparing the results with those obtained by HPLC, a higher resolution combined with a better peak shape was observed. The concentration of water in the mobile phase plays an important role in enantiomer separation. The best results were obtained using a water content of about 5%. The composition of a mobile phase was typically 90% acetonitrile, 5% acetic acid, and 5% water.

2.3. Electrochromatography with monolithic chiral stationary phases

In p-CEC, the packing bed is retained by frits at both ends of the capillary, hindering the movement of the charged particles, e.g., silica, in high electric fields. It is important that the frits are sufficiently tight to retain the particles but loose enough to permit unhindered flow. Performance and stability of packed capillaries depend on the integrity of the frits. Peak broadening in p-CEC may be caused by the inlet frit. Furthermore, the frits significantly contribute to the formation of bubbles. Problems in column packing can lead to irregularities of the packing beds which diminish the efficiency of the column. To overcome these problems, columns consisting of a block of a porous solid, called monolith or rod, prepared by in situ polymerisation or by sintering of silica, were used in LC and recently in CEC. Thus, long columns exhibiting high efficiencies can be fabricated without limitation of the packing procedure. The main problem in preparing monolithic

columns is to find conditions for the polymerization which lead to channels with good flow through properties.

Chiral monolithic separation media for CEC were prepared in two different ways: (i) a chiral print molecule is added to the polymerization mixture and removed after polymerization generating a chiral cavity (MIPs) [23,46–50] (see also Sections 2.1.4 and 2.2.7) or (ii) a direct copolymerization of a chiral selector is carried out [51].

(i) Separation systems based on continuous rods of imprinted polymers have been used for enantiomer separation of, e.g., the β-blockers propranolol, metoprolol, alprenolol, atenolol and pindolol, amino acids, and the local anaesthetics ropivacaine, mepivacaine and bupivacaine [46–50]. To avoid elution of MIPs from the capillary either by pressure or electrophoretic movement, the MIP monolith was covalently attached to the inner wall of the capillary column. The polymerization mixture typically consisted of the imprint molecule, the initiator (e.g., AIBN), the functional monomer methacrylic acid and/or 2-vinylpyridine and the cross-linker monomer (e.g., trimethylolpropane trimethacrylate). The components were filled into a pretreated capillary (e.g., by [(methacryloxy)propyl]trimethoxysilane) and the polymerization process was initiated by heating or irradiation. Schweitz et al. [46,47,49] carried out the polymerization at −20°C under a UV source. To achieve good flow-through properties, either the polymerization process was interrupted before completion [49] by removing the UV source or was carried out using a porogenic agent [47]. Using *R*-propranolol or *S*-metoprolol as imprinting molecule the enantiomer separation of *rac*-propranolol or *rac*-metoprolol were possible [49].

Later, Schweitz et al. reported on the enantiomer separation of several structural analogues of propranolol on a MIP-based column prepared with *R*-propranolol as imprinting molecule (see Fig. 20) [46]. Chiral analysis of the local anaesthetic drugs ropivacaine, mepivacaine and bupivacaine has been demonstrated on columns imprinted with *S*-ropivacaine. For successful enantiomer separation, a high concentration of organic solvent (acetonitrile) in the electrolyte was necessary [47]. Furthermore, higher temperatures (60°C) were advantageous for the separation leading to shorter migration times, higher resolution factors and higher plate numbers [47].

(ii) Peters et al. [51] prepared several monolithic chiral stationary phases for electrochromatography by direct copolymerization of the chiral monomer 2-hydroxyethyl methacrylate (*N*-L-valine-3,5-dimethylanilide) carbamate (a 'brush-type' selector) with ethylene dimethacrylate, 2-acrylamido-2-methyl-1-propanesulfonic acid and butyl or glycidyl methacrylate in the presence of a porogenic solvent. Variation of the hydrophilicity of the monolith influences enantioselectivity and efficiency of the enantiomer separation of *N*-(3,5-dinitrobenzoyl)leucine diallylamide. An efficiency of 61 000 plates/m and a resolution of 2.0 was achieved using the most hydrophilic monolithic capillary.

3. Comparison of enantiomer separation by o-CEC with p-CEC and monolithic CEC

Advantages of o-CEC are the simple preparation of the capillaries, the simple instrumental handling and the short conditioning time. Disadvantages are long migration times caused by the reduction of the EOF through coating of the internal wall and the tendency to peakbroadening as a result of overloading. The use of longer capillaries (up to 90 cm) is often necessary. Advantages of p-CEC compared to o-CEC are the higher sample capacity, shorter migration times, higher sensitivities and the use of shorter capillaries (about 15–30 cm). While its disadvantages are the tedious packing of columns, the presence of frits which can lead to bubble formation and long conditioning time. With monolithic columns, problems associated with packing and frit preparation are irrelevant. Further advantages are the possibility of use of capillaries with reduced internal diameter and the employment of microchip technology. The high stability of the columns and the short time required for preparation and conditioning are further benefits of this approach. At present, a disadvantage is the somewhat poorer efficiencies of chiral monolithic columns as compared to conventional packed columns.

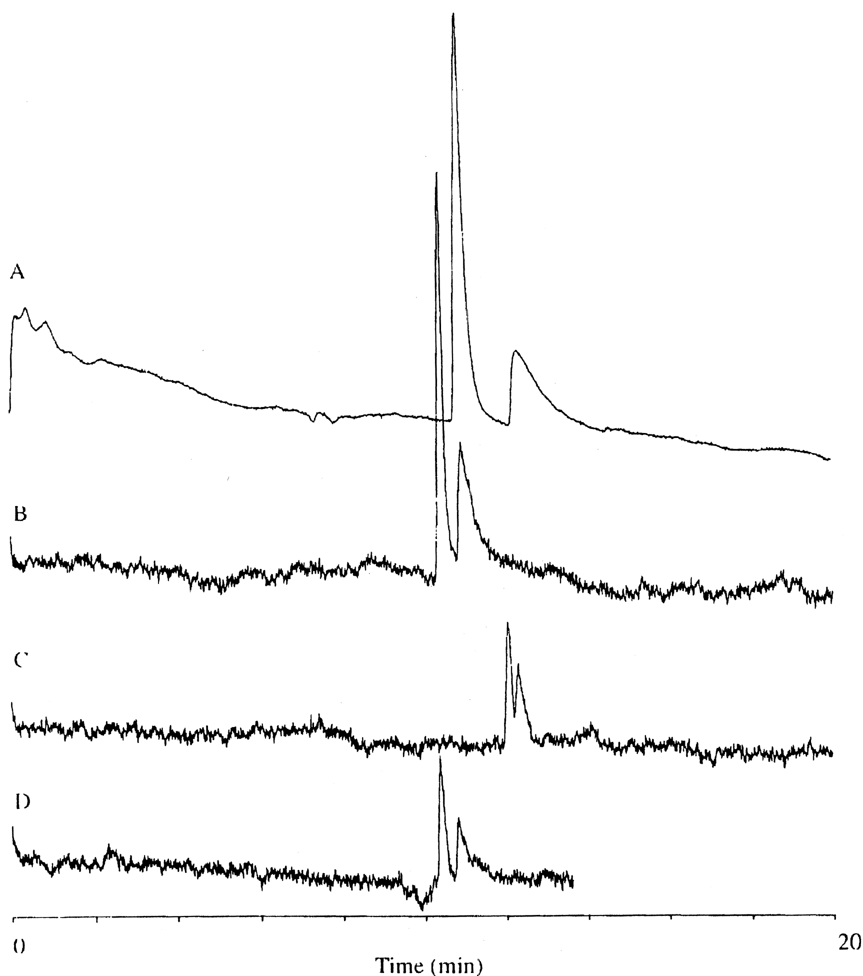


Fig. 20. Enantiomer separation of propranolol (A), pindolol (B), prenalterol (C) and atenolol (D) on a MIP-based monolithic column (90 cm×75 μm I.D.) by CEC (imprinting molecule, *R*-propranolol). Reprinted with permission from Ref. [46].

4. Conclusion and future prospects

Although a large variety of chiral compounds of pharmaceutical interest are separated into enantiomers by the classical method of HPLC, the higher efficiency and economy of electro-driven methods justifies the use of electrochromatographic techniques in routine chiral drug analysis in the future. In the last few years, the number of chiral stationary phases described for enantiomer separation in CEC is continuously increasing, but the technique of enantioselective CEC is still in a state of infancy. As robust capillaries for enantioselective CEC will become commercially available, it can be expected that the method will get a wider acceptance in the future. The full potential of p-CEC in the field of enantiomer separation has not yet been exploited: although at present electro-driven methods allow the use of very small particle sizes and long columns and thus high plate numbers because of the absence of column back pressure, typical HPLC material with particle size of 3–5 μm are still used.

For enantiomer analysis of pharmaceuticals and metabolites in biological fluids, chiral CEC–MS and chiral CEC–NMR coupling is a promising technique. In CEC, the chiral selector is incorporated into the stationary phase, and hence no problem is experienced with chiral additives proceeding in CE–

MS coupling. Further investigations will likely be concerned with temperature-dependent studies of enantioselectivity, with enantiomerization trials and miniaturization.

5. Abbreviations

AGP	α_1-acid glycoprotein
AIBN	azo-isobutyronitrile
BSA	bovine serum albumin
CE	capillary electrophoresis
CEC	capillary electrochromatography
o-CEC	open-tubular capillary electrochromatography
p-CEC	packed electrochromatography
CSP	chiral stationary phase
CZE	capillary zone electrophoresis
DNP	dinitrophenyl
EOF	electroosmotic flow
GC	gas chromatography
HPLC	high-performance liquid chromatography
HSA	human serum albumin
HTAP-β-CD	β-cyclodextrin-2-hydroxy-3-trimethylammoniumpropylether chloride
I.D.	internal diameter
o-LC	open-tubular liquid chromatography
p-LC	packed liquid chromatography
ODS	octadecylsilica
MES	2-(*N*-morpholino)ethanesulfonic acid
MIPs	molecular imprinting polymers
MS	mass spectrometry
NMR	nuclear magnetic resonance
SFC	supercritical fluid chromatography
SPE-β-CD	6-*O*-(sulfo-*n*-propyl)-β-cyclodextrin
TEAA	triethylammonium acetate
Tris	tris(hydroxymethyl)methylamine
WAX	week anion exchange

Acknowledgements

The authors thank Deutsche Forschungsgemeinschaft and Fonds der chemischen Industrie for support of this work.

References

[1] K.D. Altria, N.W. Smith, C.H. Turnbull, Chromatographia 46 (1997) 664.
[2] K.A. Kelly, M.G. Morteza, Chem. Anal. 146 (1998) 277.
[3] C. Fujimoto, Trends Anal. Chem. 18 (1999) 291.
[4] G. Gübitz, M.G. Schmid, Enantiomer, in press.
[5] V. Schurig, D. Wistuba, Electrophoresis 20 (1999) 2313.
[6] V. Schurig, M. Jung, S. Mayer, S. Negura, M. Fluck, Angew. Chem., Int. Ed. Engl. 33 (1994) 2222.
[7] V. Schurig, M. Jung, S. Mayer, M. Fluck, S. Negura, H. Jakubetz, J. Chromatogr. A 694 (1995) 119.
[8] H. Jakubetz, H. Czesla, V. Schurig, J. Microcol. Sep. 9 (1997) 421.
[9] S. Mayer, V. Schurig, J. High Resolut. Chromatogr. 15 (1992) 129.
[10] S. Mayer, V. Schurig, J. Liq. Chromatogr. 16 (1993) 915.
[11] S. Mayer, V. Schurig, Electrophoresis 15 (1994) 835.
[12] S. Mayer, M. Schleimer, V. Schurig, J. Microcol. Sep. 6 (1994) 43.
[13] D.W. Armstrong, Y. Tang, T. Ward, M. Nichols, Anal. Chem. 65 (1993) 1114.
[14] J. Sezemán, K. Ganzler, J. Chromatogr. A 668 (1994) 509.
[15] J.J. Pesek, M.T. Matyska, S. Menezes, J. Chromatogr. A 853 (1999) 151.
[16] E. Francotte, M. Jung, Chromatographia 42 (1996) 521.
[17] H. Hofstetter, O. Hofstetter, V. Schurig, J. Microcol. Sep. 10 (1998) 287.
[18] Z. Liu, H. Zou, J.Y. Ni, Y. Zhang, Anal. Chim. Acta 378 (1999) 73.
[19] Z. Liu, H. Zou, M. Ye, J. Ni, Y. Zhang, Chin. J. Chromatogr. 17 (1999) 245.
[20] Z. Liu, H. Zou, M. Ye, J. Ni, Y. Zhang, Electrophoresis 20 (1999) 2897.
[21] O. Brüggemann, R. Freitag, M.J. Whitcombe, E.N. Vulfson, J. Chromatogr. A 781 (1997) 43.
[22] Z.J. Tan, V.T. Remcho, Electrophoresis 19 (1998) 2055.
[23] L. Schweitz, L.I. Andersson, S. Nilsson, J. Chromatogr. A 817 (1998) 5.
[24] H. Jakubetz, M. Juza, V. Schurig, Electrophoresis 19 (1998) 738.
[25] H. Jakubetz, Dissertation, University of Tübingen, 1997.
[26] M. Sinibaldi, M. Vinci, F. Frederici, M. Flieger, Biomed. Chromatogr. 11 (1997) 307.
[27] S. Li, D.K. Lloyd, J. Chromatogr. A 666 (1994) 321.
[28] F. Lelièvre, C. Yang, R.N. Zare, P. Gareil, J. Chromatogr. A 723 (1996) 145.
[29] D. Wistuba, H. Czesla, M. Roeder, V. Schurig, J. Chromatogr. A 815 (1998) 183.
[30] D. Wistuba, V. Schurig, Electrophoresis 20 (1999) 2779.
[31] K. Krause, M. Girod, B. Chankvetadze, G. Blaschke, J. Chromatogr. A 837 (1999) 51.
[32] Y. Deng, J. Zhang, T. Tsuda, P.H. Yu, A.A. Boulton, R.M. Cassidy, Anal. Chem. 70 (1998) 4586.
[33] W. Wei, G. Luo, R. Xiang, C. Yan, J. Microcol. Sep. 11 (1999) 263.
[34] S. Li, D.K. Lloyd, Anal. Chem. 65 (1993) 3684.

[35] D.K. Lloyd, S. Li, P. Ryan, J. Chromatogr. A 694 (1995) 285.
[36] C. Wolf, P.L. Spence, W.H. Pirkle, E.M. Derrico, D.M. Cavender, G.P. Rozing, J. Chromatogr. A 782 (1997) 175.
[37] A. Dermaux, F. Lynen, P. Sandra, J. High Resolut. Chromatogr. 21 (1998) 575.
[38] H. Wikström, L.A. Svensson, A. Torstensson, P.K. Owens, J. Chromatogr. A 869 (2000) 395.
[39] A.S. Carter-Finch, N.W. Smith, J. Chromatogr. A 848 (1999) 375.
[40] K. Krause, B. Chankvetadze, Y. Okamoto, G. Blaschke, Electrophoresis 20 (1999) 2772.
[41] M. Lämmerhofer, W. Lindner, J. Chromatogr. A 829 (1998) 115.
[42] M. Lämmerhofer, W. Lindner, J. Chromatogr. A 839 (1999) 167.
[43] J.-M. Lin, K. Uchiyama, T. Hobo, Chromatographia 47 (1998) 625.
[44] J.-M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, J. Liq. Chromatogr. Rel. Technol. 20 (1997) 1489.
[45] J.-M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, Biomed. Chromatogr. 11 (1997) 298.
[46] L. Schweitz, L.I. Andersson, S. Nilsson, Chromatographia 49 (1999) S93.
[47] L. Schweitz, L.I. Andersson, S. Nilsson, J. Chromatogr. A 792 (1997) 401.
[48] S. Nilsson, L. Schweitz, M. Petersson, Electrophoresis 18 (1997) 884.
[49] L. Schweitz, L.I. Andersson, S. Nilsson, Anal. Chem. 69 (1997) 1179.
[50] J.-M. Lin, T. Nakagama, X.-Z. Wu, K. Uchiyama, T. Hobo, Fresenius J. Anal. Chem. 357 (1997) 130.
[51] E.C. Peters, K. Lewandowski, M. Petro, F. Svec, J.M.J. Fréchet, Anal. Commun. 35 (1998) 83.