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Review

Identification of chiral drug isomers by capillary electrophoresis

Salvatore Fanali

Istituto di Cromatografia del C.N.R., Area della Ricerca di Roma, P.O. Box 10, 00016 Monterotondo Scalo, Rome, Italy

Abstract

Separation of optical isomers of compounds of pharmaceutical interest by capillary electrophoretic techniques is reviewed. The direct and indirect separation method, as well as the main resolution mechanisms and the parameters influencing the stereoselectivity are discussed considering capillary zone electrophoresis, micellar electrokinetic chromatography, isotachophoresis and electrochromatography. Several chiral selectors have been successfully used in CE for chiral separation, including cyclodextrins and their derivatives, modified crown-ethers, proteins, antibiotics, linear saccharides and chiral surfactants. Only applications in the pharmaceutical field with the most important experimental conditions are summarised in the Tables reported in this paper. The chiral analyses of drugs in real samples like biological fluids or pharmaceutical formulations are also reported.

Keywords: Reviews; Enantiomer separation; Pharmaceutical analysis; Cyclodextrins; Drugs; Antibiotics; Crown ethers; Micelles

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1. Introduction

Many chemical compounds used in pharmaceutical formulations possess (an) asymmetric center(s) responsible for optical activity that can strongly influence their pharmacological properties. Recently it has been reported that among 523 natural and semisynthetic drugs 98.8% are chiral and 98.4% of them are sold as single enantiomer, while in the case of 1327 synthetic drugs chiral compounds represent 39.8% and only 11.6% of them are sold as single enantiomer [1]. Examples of drugs in which one of the two enantiomers possesses a different pharmacological activity are well documented, e.g., (−)-epinephrine and (−)-terbutaline are 10- and 4-times more potent than their (+)-antipode, respectively [2], (−)-propranolol is 100-fold more potent than (+)-propranolol; in addition one of the two enantiomers of the same drug can even be toxic (e.g., thalidomide, ketamine).

The demand for analytical methods with high resolution power and high efficiency is recently increasing for, e.g., chiral drug purity control, pharmacokinetic, pharmacodynamic studies, etc.

Chiral analysis is currently carried out employing chromatographic techniques including high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and gas chromatography (GC) [3–9]

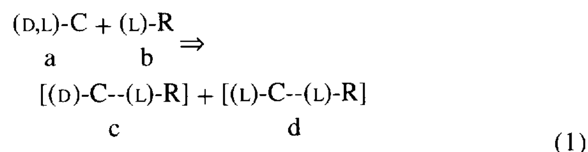
Capillary electrophoresis (CE) is a recent powerful analytical technique widely applied in different areas of research, e.g., pharmaceutical, biological, environmental, etc. The different separation modes, namely capillary gel electrophoresis (CGE), micellar electrokinetic chromatography (MEKC), isotachophoresis (ITP), capillary zone electrophoresis (CZE) and the many chiral selectors available make CE techniques a very important tool for chiral analysis in the pharmaceutical field. Compared to other analytical techniques like HPLC, CE can offer several advantages including simplicity, rapidity of analysis, automation, different separation mechanisms and low cost.

The separation of two enantiomers is a difficult task in CE because the two analytes possess similar physico-chemical properties, unless a chiral environment is used in order to selectively modify their electrophoretic mobilities. The chiral selector is usually added to the background electrolyte and only a few μl of buffer can be used for the analysis reducing the costs and environment pollution and allowing in such a way the use of even very expensive chiral selectors. Most of the work done to date concerned the separation of chiral compounds, including many drugs [10–12] using the direct separation method where different chiral selectors were added to the background electrolyte [13–16]; even chiral selectors bound to the capillary wall [17] or immobilised into a gel have been studied [18]. Several resolution mechanisms have been discussed including inclusion-complexation, metal-chiral ligand complexation, micellar solubilization, affinity and ion-pairing interactions. Even if the field of chiral analysis by CE was recently investigated, the topic has been widely reviewed [10,11,19–35].

The present paper reviews the state of the art of chiral separations performed by CE techniques focusing the attention on the identification of chiral drug isomers compounds. Different chiral resolution mechanisms are considered excluding those that were not applied to chiral drugs separation, e.g., ligand-exchange. Furthermore, selected applications in pharmaceutical and clinical fields are also discussed.

2. The indirect chiral separation method

The indirect separation method of enantiomers is based on a chemical reaction of the two antipode with a chiral compound before the electrophoretic analysis with a production of a mixture of two diastereoisomers, as shown below:



$$\underset{a}{(\mathrm{D,L})\text{-C}} + \underset{b}{(\mathrm{L})\text{-R}} \Rightarrow \underset{c}{[(\mathrm{D})\text{-C--}(\mathrm{L})\text{-R}]} + \underset{d}{[(\mathrm{L})\text{-C--}(\mathrm{L})\text{-R}]} \quad (1)$$

where C is the racemic compound, R the chiral reagent, and D- and L- are dextro and levo rotatory, respectively. The product of the reaction leads to the formation of two compounds (c and d) in which C and R are bound through covalent bonds and the configuration of R is the same in the two derivatives

and thus c and d are diastereoisomers. The mixture can be, in principle, separated by CE using an achiral background electrolyte because the components possess different physico-chemical properties.

The indirect separation method, widely used in HPLC and GC, was hardly applied in CE. This may be due to the disadvantages arising from this method in comparison to the direct separation method. Among them we can outline the following: (1) the method is time-consuming because a sample pretreatment involving a chemical reaction with a characterization of the chemical reaction products is mandatory; (2) reacting groups on the analytes chemical structure are necessary (nitrogen, hydroxyl, carboxylic); (3) one has to use very pure chiral selectors, the presence of an impure chiral selector will produce two compounds in addition to the diastereoisomers studied which makes the separation more difficult; (4) the two enantiomers should react with the same rate; (5) the response of the detector for the two diastereoisomers formed should be the same; (6) reaction conditions should be appropriate in order to avoid stereo transformation of either chiral reagent, enantiomers or diastereoisomers.

Very often it is not easy to resolve all the operational problems described above. However, note that the indirect method can give a valid solution in those cases where the direct method cannot be used or when the detectability of analytes should be increased.

The indirect separation method was mainly applied in CE for the enantiomeric resolution of amino acids using different chiral selectors, e.g., 2,3,4,6-tetra-O-acetyl-D-glucopyranosyl isothiocyanate (GITC) [36], (+)-O,O′-diacetyl-L-tartaric anhydride or (+)-O,O′-dibenzoyl-L-tartaric anhydride [37,38], 1-fluoro-2,4-dinitrophenyl-5-L-alanine or 1-fluoro-2,4-dinitro-5-D-alanine (L- or D-Marfey's reagent) [39]. However the applicability of this method has also been shown for compounds of pharmaceutical interest by Dette and Watzig [40,41].

Enantiomer separation of amphetamine, methamphetamine, ephedrine, pseudoephedrine, norephedrine and norpseudoephedrine in a single MEKC run after derivatization with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) was described by Lurie [42]. The effect of organic modifier type and concentration, SDS concentration, capillary temperature and the applied voltage on the resolution and selectivity were studied. The optimum experimental conditions for the enantiomer separation of 12 phenylethylanine isomers were found to be at 30°C with a background electrolyte (BGE; here 10 m*M* phosphate+10 m*M* borate buffer pH 9) containing 100 m*M* SDS and 20% methanol. The applicability of the optimised method was shown analyzing a forensic sample containing 1*R*,2*S*-(−)-ephedrine and 2*S*-(+)-methamphetamine.

One interesting example where the direct resolution method is difficult to apply is represented by the chiral separation of L- and D-carnitine. The L-carnitine enantiomer is very important in the metabolism of long fatty acids, while the D-isomer plays a toxic effect on biochemical processes, thus the drug should not contain D-carnitine. The separation of D- and L-carnitine has been performed after derivatization with (−)-[1-(9-fluorenyl)-ethyl]chloroformate (FLEC) in 50 m*M* phosphate buffer pH 2.6. The addition of 20 m*M* tetrabutylammonium bromide improved the diastereoisomer resolution [43].

Recently the indirect chiral separation method has been used for the enantiomer separation of several amphetamines such as amphetamine, 4-hydroxyamphetamine, 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyethamphetamine after derivatization with Marfey's reagent. The BGE was 80% aqueous buffer (5 m*M* borate pH 9 and 100 m*M* SDS) and 20% methanol [44].

3. The direct chiral separation method

The direct chiral separation method is successfully applied in CE for the enantiomeric separation of several classes of compounds, mainly, of pharmaceutical interest. The chiral selector can either be added to the background electrolyte [13–15,22] or bound to the capillary wall [17], or included in/bound to a gel matrix [18,45] in order to arrange a stereoselective environment that is interacting, during the electrophoretic run, with the two enantiomers on forming labile diastereoisomeric complexes. The labile complexes formed are moving toward the detector at a different velocity only if they possess different stability constants. In this case relatively weak bonds are involved in the diastereoisomer

formation process (hydrogen, hydrophobic, $\pi-\pi$, dipole–dipole).

The chiral purity of the selector in the direct separation method is not as critical a factor as in the indirect separation method; in fact, the impurity is influencing only the resolution and it has been shown that relatively good results can be obtained using a chiral selector containing up to 10% of its antipode [11].

Several resolution mechanisms and the main parameters influencing the selectivity of the chiral separation will be discussed including inclusion-complexation, micellar and affinity interactions

3.1. Inclusion-complexation

The inclusion-complexation mechanism has been widely applied in analytical chemistry in order to improve the selectivity of the separation of several isomeric compounds including geometrical, positional and enantiomeric. Up to now three classes of compounds, forming inclusion-complexes, have been used in CE, namely cyclodextrins (CDs), tetracarboxylated crown-ether and antibiotics.

3.1.1. The use of cyclodextrins or their derivatives

CDs are oligosaccharides composed by different glucose units connected to each other through α(1,4)-glucosidic bonds and they are obtained by enzymatic reaction with starch. In spite of the fact that CDs formed from 6 to up to 12 glucose units have been isolated, only those with six, seven and eight units are currently used, termed α-, β- and γ-CD, respectively. The CD's shape is similar to that of a truncated cone with a relatively hydrophobic cavity and able to host analytes, and an hydrophillic outside region due to the presence of hydroxyl groups (position 2, 3 and 6 of glucopyranose). The three native cylodextrins possess the same depth but have different widths (increasing by the number of glucose units).

In the inclusion-complexation mechanism the compound fits the CD cavity with the whole molecule or with its hydrophobic part and, thus, the CD type has a very important role in the separation process. The hydrophobic interaction with the cavity alone is not sufficient to enable the separation of enantiomer compounds; weak bonds between sub-

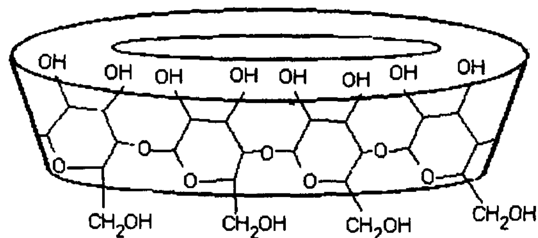


Fig. 1. Structure of CD.

stituent groups on the asymmetric center/s of analytes and secondary and/or primary hydroxyl groups of the CD are responsible for chiral recognition.

Fig. 1 shows the shape of a CD while Table 1 shows the main properties of the native CDs used in CE.

Due to its dimensions β-CD is effective for the inclusion complexes with molecules composed of one or two condensed aromatic groups, and the modification of hydroxyl groups at position 2, 3 and 6 of glucopyranose after chemical reactions enormously extends the use of such CD types in chiral CE analysis. The modified CDs can exhibit very different properties than the parent ones, which can be useful for improving the selectivity in chiral analyses, e.g., higher solubility, different conformation, charged or chargeable groups, etc. The case of heptakis-(2,6-di-O-methyl)-β-CD can serve here as an example. The two hydroxyl groups at position 2 and 6 of each glucose is modified with methoxy groups and as a result the oligosaccharide possesses a deeper cavity and an higher solubility than β-CD.

CDs and their derivatives have been widely applied in CE for the enantiomer separation of a large

Table 1
The main properties of native CDs (see Ref. [46])

	CD type		
	α	β	γ
Number of glucopyranose units	6	7	8
Molecular mass (Da)	972	1135	1297
Inner diameter (nm)	0.57	0.78	0.95
Depth (nm)	0.78	0.78	0.78
Solubility in water (g/100 ml)	14.5	1.85	23.2
$[\alpha]_D^{25}$	+150.5	+162.0	+177.4

number of compounds of pharmaceutical interest used as standards with the aim to study the parameters influencing the selectivity of the separation.

The first attempt to perform chiral separation by electromigration methods was done by Smolkova's group by ITP employing dimethylated (di-OMe-β-CD) or trimethylated-β-CD (tri-OMe-β-CD) as chiral selector added to the leading electrolyte [47]. After studying different operational parameters, e.g., CD type and concentration, the enantiomer resolution of racemic standard drugs, including pseudoephedrine, norpseudoephedrine, O-acetylpseudoephedrine and p-hydroxynorpseudoephedrine was performed. This is not the only example of chiral drug analysis by ITP, several others separations carried out by the same group document the applicability of ITP to enantiomer separation, e.g., ketotifen drug and its polar synthesis intermediates were resolved in their enantiomers using β-CD or di-OMe-β-CD [48]. Furthermore, a coupled column system, in which the two capillaries contained two different CDs, has been discussed [49]. Comparison of ITP and CZE for enantiomer separation of pharmaceutical compounds (chloramphenicol, ketotifen, ketotifen-N-oxide and thioridazine) was also discussed [50]; the authors concluded that ITP was more suitable than CZE for the analysis of minute components in a large excess of others but CZE is preferable when high efficiencies are requested. Theoretical models for enantiomer separation using ITP have also been discussed [51,52]. Unfortunately, after these excellent results ITP was not used for further investigations in chiral drug analysis.

CZE has been tested for chiral separations of amino-acid derivatives using ligand exchange mechanism [53,54] or CDs incorporated into a gel [18] or charged CDs [13]. The first attempt to use CZE for the enantiomer separation of compounds of pharmaceutical interest was done by us [14] studying several sympathomimetic drugs, namely ephedrine, norephedrine, epinephrine, norepinephrine and isoproterenol. Among the CDs studied di-OMe-β-CD was the most effective in chiral separation performed in a short capillary (20 cm) coated with linear polyacrylamide. The increase in CD concentration led to an increase in migration time and resolution. The effect of the concentration of CDs on the enantioselectivity was discussed by several authors indicating that this parameter should be carefully controlled for the success of chiral drug separation [15,55–57]

As mentioned above the selection of the appropriate CD is of paramount importance when chiral drug separations have to be performed. As first requirement for chiral resolution using CDs, the analytes must fit the cavity when forming inclusion complexes and thus the dimension of the chiral selector must be appropriate. Nardi et al. [58] separated racemic isolysergic acid, meluol, terguride, lisuride and nicergoline, ergot alkaloid drugs, only using γ-CD with the largest cavity. The enantiomer separation of terbutaline was performed using both β-CD and di-OMe-β-CD; the modified CD allowed to obtain a higher enantioselectivity (maximum resolution at 5 mM) than the parent one (maximum resolution at 15 mM). These results can be explained considering that the modified CD possesses a deeper cavity (more hydrophobic) than β-CD and modified hydroxyl groups at position 2 and 6 that can influence secondary bonds [59].

The modification of the hydroxyl groups on the CD rims is of primary importance in order to improve the stereoselectivity of the separation because these groups are forming secondary bonds with the enantiomers. Therefore, attention should be paid to the degree of substitution of the CD. This effect was studied by Valko et al [60] using HP-β-CD at different degree of substitution for the chiral separation of several organic acids and by Yoshinaga and Tanaka [61] using different methylated β-CD for the chiral resolution of some dansyl-amino acids. Recently we studied the effect of different CD and their concentration on the enantiomers resolution of some non-steroidal anti-inflammatory drugs (NSAIDs), namely fenoprofen, flurbiprofen, ketoprofen, ibuprofen, indoprofen and suprofen. Among the CDs used (di-OMe-β-CD, tri-OMe-β-CD, 6^{A}-methylamino-β-CD) tri-OMe-β-CD proved to be the most effective chiral selector allowing the base line separation of all the enantiomers studied [62].

Both native and derivatised CDs have been investigated by Altria et al. [15] for chiral separation of pharmaceutical compounds by CZE. Picumeterol and clenbuterol, two β-agonistic drugs were resolved in their enantiomers and the effect of several parameters, e.g., pH and ionic strength of the BGE, CD type

and concentration, capillary temperature on enantioselectivity were investigated. The capillary temperature can strongly affect the resolution (generally a lower temperature increases resolution) as also discussed by Schutzner and Fanali [63] studying the chiral separation of some sympathomimetic drugs, namely norepinephrine, epinephrine and isoproterenol. Increasing the column temperature caused a decrease of both migration time and resolution. The decrease of enantioselectivity can be ascribed to the shorter time spent by the analytes into the CD cavity and to a modification of thermodynamic parameters. The effect of the pH on the enantiomer separation was studied by Palmarsdottir and Edholm [55] and the resolution of terbutaline decreased by increasing the pH (2.5–8) when di-OMe-β-CD was used.

Enantioselectivity, efficiency and resolution can also be influenced modifying other parameters such as the organic additive as shown by us for the separation of propranolol. 30% (v/v) methanol was the additive contained in phosphate buffer–urea and 40 mM of β-CD [59]. Recently similar effect was noticed for the chiral separation of racemic flurbiprofen (see Fig. 2); here the analyte showed poor resolution at 5 mM of tri-OMe-β-CD (very good resolution was obtained at 30 mM of CD without organic additive) but the addition of methanol to the BGE (MES at pH 5) caused an increase of resolution [62]. The effect of organic modifier on enantiomers resolution using CDs as chiral selector was theoretically discussed by Wren [64], who showed that when the CD concentration was at or below the optimum value (maximum resolution), the addition of organic modifier caused a reduction of resolution due to the change of stability constants of the two complex CD-enantiomers.

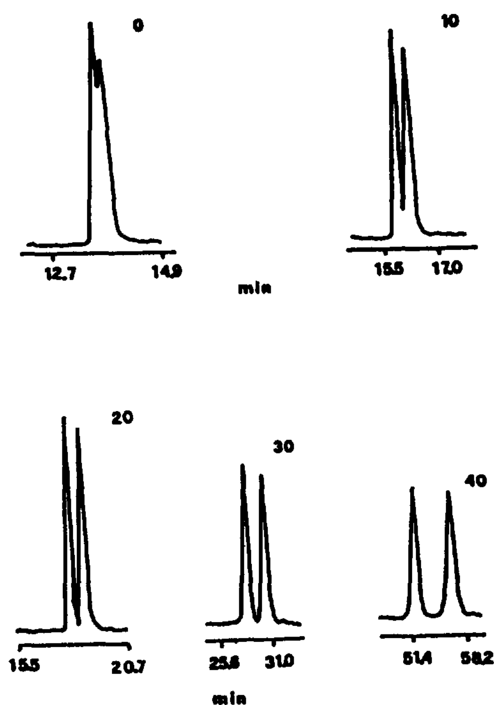


Fig. 2. Electropherograms of the enantiomer separation of racemic flurbiprofen using background electrolytes at pH 5 containing 5 mM of tri-OMe-β-CD and different concentrations of methanol (0–40%). Apparatus, Biofocus 3000 (reprinted with permission from Ref. [62]).

Manipulation of the BGE for improving enantioselectivity and efficiency of chiral drug separations can also be done using alkylhydroxyalkylcellulose derivatives added to the BGE containing CDs, as shown for drugs like threo-chloramphenicol and ketotifen [50]; hydroxyethyl cellulose was added to the BGE containing di-OMe-β-CD. Similar effect was recorded by Belder and Schomburg [65] for the analysis of tocainide analogues using polyvinyl alcohol (PVA). The polymer used reduced the electro-osmotic flow, as well as the adsorption on the capillary wall causing an improvement in enantioselectivity. Recently it has been shown that the addition of short chain tetra alkyl ammonium cations (TAA) to the chiral BGE can strongly affect the selectivity of the enantiomer separation of several racemic drugs [66]. TMA and TBA were added to the BGE at pH 2.5 containing different CDs (β-CD, di-OMe-β-CD, tri-OMe-β-CD and HP-β-CD) for the enantiomer separation of 22 compounds of pharmaceutical interest. The use of TAA caused the reversion of the electro-osmotic flow, a generally increase of either migration time and stereoselectivity was recorded. Compared to TMA, TBA provided a different selectivity increasing the solubility of β-CD and lower conductivity, however in some extent a reduction of resolution was recorded due to the competition of this cation for the CD cavity. To illustrate this, Fig. 3 shows the effect of TMA and TBA on the enantiomer separation of trimipramine [66].

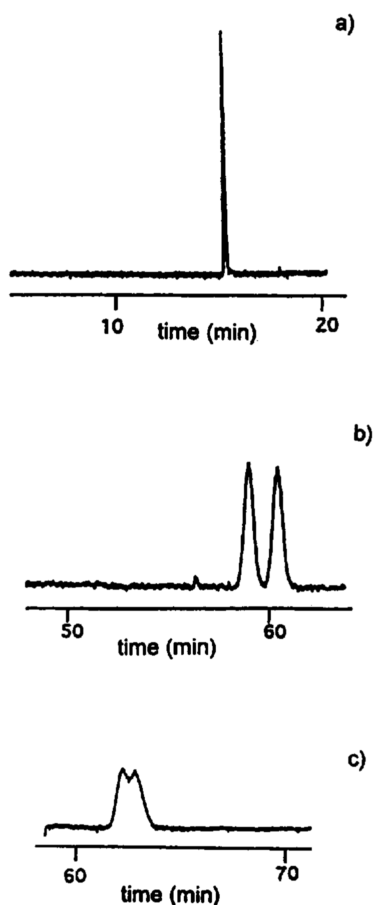


Fig. 3. Effect of TMA and TBA on the enantiomer separation of trimipramine. Background electrolytes: 20 m*M* HP-β-CD + (a) 50 m*M* sodium phosphate pH 2.5; (b) 100 m*M* TBA–phosphate pH 2.5; (c) 50 m*M* TMA pH 2.5 (modified from Ref. [66])

Reversal of the electro-osmotic flow was also used by Stalberg et al. [67] for improving the enantiomer separation of several local anaesthetic drugs, with tetrapropylammonium (TPA) and TBA being added to the BGE at pH 3 containing di-OMe-β-CD

Recently uncharged β-CD polymer has been studied for chiral drug separation in CE. The polymer contains several β-CD units bound to each other after reaction with epichloroydrin and, thus, inclusion-complexation interaction alone cannot explain the stereoselectivity.

Nishi et al. [68] separated several trimetoquinol analogues, bronchodilator drugs, using 7% of β-CD polymer with a BGE at pH 6.5. Recently we investigated the effect of the uncharged β-CD polymer on the enantiomer separation of a large number of drugs, including β-blockers, α- and β-antagonists, and anaesthetics [69,70]. Increasing the concentration of chiral polymer a general improvement of the enantiomer resolution was recorded while the addition of organic additive caused a decrease in resolution, except for propanolol and ephedrine. The same chiral selector was employed by Nishi et al. [71] for the chiral purity control of several drugs such as trimetoquinol, denopamine and trimepidium. The CE method was shown to be very reproducible and useful for quantitative chiral analysis. To illustrate this, Fig. 4 shows the electropherogram of the enantiomer separation of trimetoquinol and denopamine where 0.2% and 0.5% of minor isomer was detected.

Many imidazole derivatives are currently used as antimycotics, antineoplastic agents, antiepiletics, etc., and apart from this some imidazole and triazole derivatives have been separated in their enantiomers by CE [56,72–74]. Recently, enantiomer separation of pharmaceutical compounds containing imidazole moiety was systematically studied by CE using different CDs [75]. Native (α-, β- and γ-CD) and derivatised CDs (HP-β-CD) were tested for the chiral resolution of racemic bifonazole, ornidazole, econazole, miconazole, enilconazole, ketoconazole, metomidate and lofexidine. β-, γ- or HP-β-CD were able to resolve most of the studied compounds. The chiral separation was influenced not only by the CD type but also by the chiral selector concentration and by the BGE's pH and the organic additive. Enilconazole could be resolved in its enantiomers at pH lower than 6–7 using uncharged CDs, the optimum experimental conditions were found at pH 3 with 2.5 m*M* β-CD and 10% methanol.

As mentioned before, to a cetain extent it could be advantageous to derivatize the two enantiomers before the CE analysis using an achiral reagent with the aim to either improve the detectability or to introduce groups that can easily interact with the chiral selector present in the electrophoretic separation system. This interesting approach has been shown for the separation of D- and L-carnitine that due, to the chemical structure, were not separated from each other without derivatization. Here the two enantiomers are injected for the CE analysis after derivatization with an achiral reagent, 9-fluorenyl-

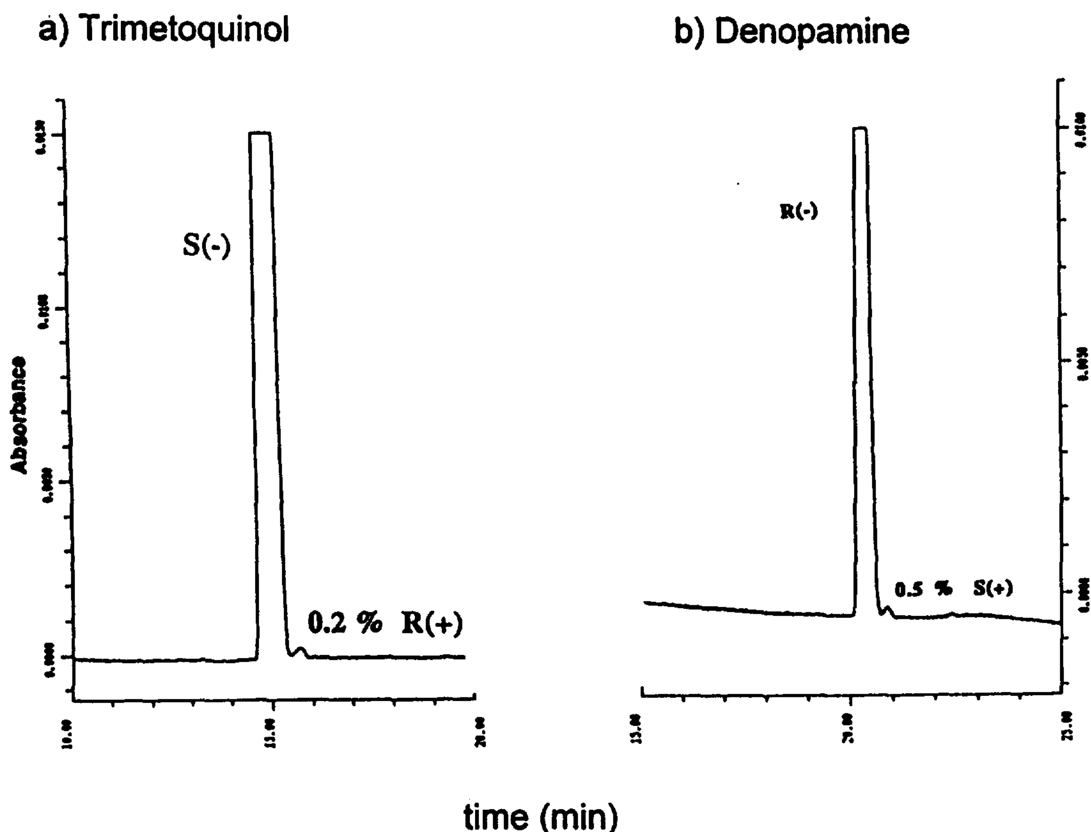


Fig. 4. Electropherogram of the enantiomer separation of trimetoquinol and denopamine using uncharged β-CD polymer and di-OMe-β-CD. Background electrolytes: (a) 25 m*M* phosphate buffer pH 2.7 and 5% β-CD polymer (20 kV applied voltage); (b) 25 m*M* phosphate buffer pH 2.7, 2 *M* urea and 20 m*M* di-OMe-β-CD (15 kV applied voltage); capillary, 57(50) cm × 75 μm I.D. (modified from Ref. [71])

methylchloroformate. The use of phosphate buffer at pH 2.6 containing TBABr and γ-CD allowed the enantiomers resolution of carnitine derivatives [43]

Optimization studies have been investigated developing a theoretical model where the electrophoretic mobility was correlated to the CD concentration. An optimum CD concentration was found for propranolol using di-OMe-β-CD as chiral selector [76]. The same model was applied for the enantiomer separation of oxprenolol and metoprolol [77], for practolol, ephedrine and atenolol [64]. Penn et al. calculated the stability constants of tioconazole enantiomers with HP-β-CD and the optimum resolution experimental conditions were predicted. The electrophoretic mobility were corrected for the effect of the change of viscosity due to the increase of the CD concentration. A more complex theoretical model studying the effect of pH and concentration of β-CD on chiral selectivity for fenoprofen and ibuprofen was discussed by Vigh's group [78]. The same group used HP-β-CD in CE for the study of the effect of pH and CD concentration on peak resolution of enantiomers of atropine (desionoselective separation), chloroamphetamine (ionoselective separation) and propranolol (duoselective separation). Peak resolution surfaces were calculated and used for the analysis of the possibility of chiral separations [79]. The theoretical approach was also used for weak acids like naproxen and fenoprofen concluding that the success of the chiral separation was primarily influenced by the pH of the BGE and secondarily by the concentration of the chiral selector [80].

Other manipulations of the BGE for improving the selectivity of the enantiomer separation include the

addition of achiral surfactants to the BGE containing CDs. This method proved to be effective mainly for the resolution of uncharged compounds.

The electrophoretic system is composed of ionic surfactants forming micelles (SDS is the most used) and an aqueous phase (buffer combined with CDs). The uncharged analytes are moving with the electroosmotic flow and a repartition equilibrium is established between the micellar-aqueous phases causing a different velocity of analytes. The chiral separation is due to the CD that will cause a lowering of the migration times of the two enantiomers depending from the CD-analyte complexation.

Several parameters can be modified for improving the stereoselectivity when CD-MEKC is used, e.g., surfactant type and concentration, CD type and concentration, buffer type, ionic strength, pH and organic additive.

CD-MEKC has been widely employed for the enantiomer separation of derivatised amino acids. Dansyl-amino acids have been resolved using SDS and γ-CD [81] or a mixture of γ- and β-CD, while for naphthalene-2,3-dicarboxaldehyde (CBI-amino acids) γ-CD/SDS proved to be effective [82,83]. The applicability of CD-MEKC for the chiral separation of pharmaceutical compounds has been shown by Nishi et al. [84], who showed the separation of thiopental and pentobarbital in their enantiomers at pH 9 in the presence of SDS and γ-CD; the enantioselectivity was improved by adding *l*-methoxyacetic (l-men) or *d*-camphor-10-sulfonic acids (d-cam) to the BGE. The enantiomer separation of pharmaceutical compounds by CD-MEKC includes cycletanine [85], diniconazol and uniconazol [56,86], thiazole derivatives [87], hexobarbital, mephobarbital, secobarbital, gluthetimide, glutethimide analogues and fadrozole [88]. Different CDs in combination with SDS have been tested for the enantiomer separation of mephenytoin, phenytoin and their 4-hydroxy derivatives and the use of β-CD allowed the complete resolution of the studied compounds [89]. Recently modified charged and non-charged β-CD have been used in micellar system at pH 9.5 containing either SDS or STDC for the chiral resolution of several racemic compounds including β-agonists, β-antagonists, phenylethylamine stimulants and the antidepressant diclofensine [90].

The combination of β-CD and STDC was also used for the chiral separation of mephenytoin and hydroxymephenytoin [91], as well as for the separation of baclofen and its aminophosphoric analogues [91].

A comparison of the different CE modes, namely CZE and MEKC has been shown by Anigbogu et al. [92] for the enantiomer separation of aminoglutethimide, a drug used for the treatment of adrenocortical tumours. At pH 9, where the analytes are neutral, the combination of β-CD and CM-β-CD in the presence of 50% MeOH enabled the base-line separation. Good results were also obtained at pH 3 using CZE; α-, as well as γ-CD allowed the chiral resolution.

Recently enantiomer separation of oxamniquine by CE was compared with the results obtained using HPLC [93]. The chiral stationary phase (Cyclobond I and II) used in HPLC did not allow the enantiomer resolution of the studied compound, while a chiral-AGP protein stationary phase allowed to obtain successful chiral separation, but the results were strongly influenced by small changes of pH (0.2 units). β-CD added to the BGE at pH 12 showed that CE can give better results than HPLC; in fact, good results were obtained, even if the pH was changed 1 pH unit. In a recent study Piperaki et al. [94] investigated the enantiomer separation of fluoxetine and norfluoxetine using HPLC and CE. The binding constants were measured with both techniques under the same experimental conditions, giving good agreement between the data obtained. Binding constants were influenced by the CD type, ionic additives and organic solvents. It was concluded that CE can be used advantageously in order to predict the useful organic solvent for HPLC.

Trimepidium, trimetoquinol and denopamine enantiomers were successfully separated using CE and HPLC with the direct separation method. CE proved to be useful for quality control of chiral drugs and it was possible to detect as little as 0.1% of inactive enantiomer using CE [71].

Recently, ion-spray mass spectrometry coupled with CE was used for monitoring the enantiomer separation of terbutaline and ephedrine. The system was able to detect both free enantiomers and enantiomer-CD complexes for terbutaline. The method was shown to be suitable for the analysis of terbutaline enantiomers spiked into a urine sample and the MS

Table 2
The enantiomers separation of compounds of pharmaceutical interest using uncharged native and derivatized cyclodextrins

Compounds	CE type	CD	BGE	References
Aldose reductase inhibitor (AL03152)	CZE	β-CD or di-OMe-β-CD or HE-β-CD or HP-β-CD	20 m*M* Tris–10 m*M* H_3PO_4 pH 11 and 9 m*M* C.S.	[96]
Alprenolol	CZE	HP-β-CD	50 m*M* phosphate pH 2.5, 70 m*M* TMA and 20 m*M* C.S.	[97]
	CZE	di-OMe-β-CD	100 m*M* phosphoric acid–triethanolamine pH 3, 30% MeOH and 15 m*M* C.S.	[98]
	CZE	β-CD, di-OMe-β-CD, HP-β-CD	50 m*M* TMA–phosphate pH 2.5 and 20 m*M* C.S.	[66]
Ambucetamide	CZE	HP-β-CD	50 m*M* phosphate pH 3.3 and 30 m*M* C.S.	[72]
Aminoglutethimide	CZE	α-CD or γ-CD	$NaH_2PO_4/Na_2B_4O_7$ pH 7 and 10 or 5 m*M* C.S.	[92]
aminoglutethimide analogue	CZE	γ-CD or di-OMe-β-CD	50 m*M* sodium phosphate pH 2.5 and 15 m*M* C.S.	[88]
Amphetamine	CZE	di-OMe-β-CD	98.8% (25 m*M* Tris–H_3PO_4 pH 2.45 and 5 m*M* C.S.), 1.2% MeOH	[99]
	CZE	β-CD polymer	50 m*M* phosphate pH 2.5 and 200 mg/ml C.S.	[69]
Amphetamine and related drugs (methamphetamine, 4-hydroxy-amphetamine, 4-methoxy-amphetamine, 3,4-methylene-methamphetamine, 3,4-methylenedioxy-ethamphetamine, 2,5-dimethoxy-amphetamine, 2,5-dimethoxy-methamphetamine, 4-bromo-2,5-dimethoxy-amphetamine)	CZE	β-CD or di-OMe-β-CD, tri-OMe-β-CD, HP-β-CD	50 m*M* dihydrogenphosphate pH 2.5 and 10 m*M* C.S.	[44]
Atenolol	CZE	M-β-CD or di-OMe-β-CD	20 m*M* Tris–H_3PO_4 pH 2.4 and 24 or 28 m*M* C.S.	[96]
	CZE	M-β-CD	40 m*M* Li phosphate pH 3 and 37 m*M* C.S.	[77]
	CZE	di-OMe-β-CD	50 m*M* Li phosphate pH 2.5 and 40 m*M* C.S.	[64]
	CD-MEKC	β-CD	40 m*M* borate pH 9.3, 32 m*M* SDS and 12 m*M* C.S.	[100]

	CZE	di-OMe-β-CD	100 mM phosphoric acid–triethanolamine pH 3 and 30 mM C.S.	[98]
	CZE	di-OMe-β-CD, HP-β-CD	50 mM TMA–phosphate pH 2.5 and 20 mM C.S.	[66]
	CZE	β-CD polymer	50 mM phosphate pH 2.5 and 100 mg/ml C.S.	[69]
Atropine	CZE	HP-β-CD	100 mM TAPS pH (8–10) and C.S. (15–100 mM)	[101]
β-Agonist, β-antagonists (clenbuterol, cimaterol, terbutaline, salbutamol, alprenolol, pindolol, oxprenolol, epinephrine)	CZE	HP-β-CD	100 mM citric acid–19.27 mM Na_2HPO_4 pH 2.5 and 120 mM C.S.	[102]
β-agonists and β-antagonists (timolol, nadolol, oxprenolol, cimaterol, clenbuterol, diclofensine, pindolol, metoprolol)	CD-MEKC	HP-β-CD	50 mM borate pH 9.5, 5% propan-1-ol, 50 mM STC and 60 or 120 mM C.S.	[90]
Baclofen (CBI)	CD-MEKC	β-CD	30 mM Na_2HPO_4, 10 mM boric acid pH 7, 50 mM STDC and 20 mM C.S.	[103]
Bambuterol	CZE	di-OMe-β-CD or tri-OMe-β-CD	50 mM phosphate pH 2.5 and 15 or 5 mM C.S.	[55]
	CZE	HP-β-CD	phosphate pH 3 (I=0.05), 20 mM TBA and 60 mM C.S.	[67]
BCH 189	CZE	di-OMe-β-CD	100 mM NaH_2PO_4–H_3PO_4 pH 2.3 and 50 mM C.S.	[104]
Benzetimide	CZE	HP-β-CD	citrate–NaOH–HCl (Merck 9435) pH 4 and 10 mM C.S.	[105]
Bupivacaine	CD-MEKC	di-OMe-β-CD	18 mM Tris–H_3PO_4 pH 2.9, 0.1% MHEC, 0.03 mM HTAB and 10 mM C.S.	[106]
	CZE	di-OMe-β-CD	100 mM phosphoric acid–triethanolamine pH 3 and 30 mM C.S.	[98]
	CZE	β-CD polymer	50 mM phosphate pH 2.5 and 200 mg/ml C.S.	[69]
Butabarbital	CD-MEKC	β-CD	20 mM NaH_2PO_4–20 mM sodium tetraborate pH 7, 100 mM SDS, 2 M urea, 15% MeOH and 30 mM C.S.	[88]
Carbinoxamine	CZE	β-CD	100 mM phosphate pH 2.5, 1.5 M urea and 30 mM C.S.	[107]
Carvedilol	MEKC	di-OMe-β-CD	18 mM Tris–H_3PO_4 pH 2.9, 0.1% MHEC, 0.03 mM HTAB and 10 mM C.S.	[106]
	CZE	β-CD	50 mM phosphate pH 3.3 and 16.3 mM C.S.	[72]
Cathinone	CZE	di-OMe-β-CD	90% (25 mM Tris–H_3PO_4 pH 2.45 and 5 mM C.S.), 10% MeOH	[99]
Chloramphenicol	CZE	di-OMe-β-CD	20 mM Tris–citric acid pH 3.5, 0.1% HEC and 10 mM C.S.	[50]

(Continued on p. 88)

Table 2 (continued)

Compounds	CE type	CD	BGE	References
Chlorpheniramine	CD-MEKC	β-CD	pH 3, 5 *M* urea, 50 m*M* SDS and 100 m*M* C.S.	[108]
	CZE	β-CD	100 m*M* phosphate pH 2.5, 10% MeOH, 1.5 *M* urea and 30 m*M* C.S.	[107]
	CZE	HP-β-CD or β-CD	100 m*M* phosphoric acid–triethanolamine pH 3 and 30 or 15 m*M* C.S.	[98]
	CZE	β-CD, di-OMe-β-CD, tri-OMe-β-CD, HP-β-CD	50 m*M* TMA–phosphate pH 2.5 and 20 m*M* C.S.	[66]
Cicletanine	CD-MEKC	γ-CD	100 m*M* borate buffer pH 8.6, 110 m*M* SDS, 10% ACN and 25 m*M* C.S.	[85]
Clenbuterol	CZE	HP-β-CD	50 m*M* phosphate pH 3.3 and 30 m*M* C.S.	[72]
	CZE	β-CD	200 m*M* Na_2HPO_4–100 m*M* citric acid pH 4 and 16 m*M* C.S.	[15]
	CZE	HP-β-CD	50 m*M* sodium tetraborate–phosphoric acid pH 2.2 and 30 m*M* C.S.	[109]
	CZE	β-CD	phosphate buffer pH 2.5, 4, 5.5, MeOH 0–8% and 6–16 m*M* C.S.	[110]
	CZE	HP-β-CD	phosphate pH 3 (I=0.05), 20 m*M* TBA and 60 m*M* C.S.	[67]
	CZE	β-CD polymer	50 m*M* phosphate pH 2.5 and 100 mg/ml C.S.	[69]
Cocaine	CZE	di-OMe-β-CD	98.8% (25 m*M* Tris–H_3PO_4 pH 2.45 and 5 m*M* C.S.), 1.2% MeOH	[99]
Denopamine	CZE	di-OMe-β-CD	25 m*M* phosphate pH 2.7, 2 *M* urea and 20 m*M* C.S.	[71,111,112]
Deprenyl and its main alkaline metabolites (amphetamine, methamphetamine, propargylamphetamine)	CZE	di-OMe-β-CD	20 m*M* Tris–phosphate, 0.1% or 0.5% HPMC and 6 m*M* C.S.	[113]
Dihydropyridine derivative	CZE	HP-β-CD	10 m*M* citric acid–Tris pH 6 and 0.3% C.S.	[114]
	CZE	HP-γ-CD	20 m*M* phosphate pH 6 and 0.5% C.S.	[114]
Dimethindene	CZE	HP-β-CD	50 m*M* phosphate pH 3.3 and 30 μg/ml C.S.	[72]
	CZE	HP-β-CD	100 m*M* phosphoric acid–triethanolamine pH 3 and 30 m*M* C.S.	[98]
Diniconazole	CD-MEKC	γ-CD or di-OMe-β-CD	100 m*M* borate pH 9, 100 m*M* SDS, 2 M urea and 50 m*M* C.S.	[56,86]
Ephedrine	CZE	di-OMe-β-CD	10 m*M* Tris–phosphate pH 2.4 and 20 m*M* C.S.	[14]
	CZE	di-OMe-β-CD	50 m*M* phosphate pH 3.3 and 30 m*M* C.S.	[72]

	CZE	β-CD	150 mM TBA–phosphate pH 2.5 and 20 mM C.S.	[115]
Ephedrine	CZE	di-OMe-β-CD	50 mM Li phosphate pH 2.5 and 50 mM C.S.	[64]
	CZE	di-OMe-β-CD	30 mM Tris–phosphate pH 2.5 and 40 g/l C.S.	[116]
	CZE	di-OMe-β-CD	20 mM phosphate pH 2.5, 10 mM TBAB, 0.1% HPC and 18 mM C.S.	[117]
	CZE	di-OMe-β-CD or HP-β-CD	100 mM phosphoric acid–triethanolamine pH 3 and 30 mM C.S.	[98]
	CZE	di-OMe-β-CD	50 mM phosphate pH 2.5 and 15 mM C.S.	[55]
	CZE	β-CD, di-OMe-β-CD, HP-β-CD	50 mM TMA–phosphate pH 2.5 and 20 mM C.S.	[66]
	CZE	di-OMe-β-CD	20 mM phosphate pH 2.5 and 20 mM C.S.	[118]
Epinephrine	CZE	di-OMe-β-CD	10 mM Tris–phosphate pH 2.4 and 20 mM C.S.	[14]
	CZE	di-OMe-β-CD	100 mM phosphate pH 2.5 and 20 mM C.S.	[119]
	CZE	di-OMe-β-CD	50 mM phosphate pH 2.5 and 20 mM C.S.	[63]
	CZE	di-OMe-β-CD	100 mM NaH_2PO_4 pH 2.5, 10% MeOH and 30 mM C.S.	[107]
	CZE	di-OMe-β-CD, M-β-CD	20 mM Tris–H_3PO_4 pH 2.3 and 9 mM C.S.	[96,120]
	CZE	β-CD	150 mM phosphate–TBA pH 2.5 and 20 mM C.S.	[115]
	CZE	β-CD, di-OMe-β-CD, HP-β-CD	50 mM TMA–phosphate pH 2.5 and 20 mM C.S.	[66]
	CZE	β-CD polymer	50 mM phosphate pH 2.5 and 100 mg/ml C.S.	[69]
Etilefrin	CZE	HP-β-CD	50 mM phosphate pH 3.3 and 30 mM C.S.	[72]
	CZE	di-OMe-β-CD	25 mM phosphate pH 3, 2 M urea	[111]
Etodolac	CZE	M-β-CD or γ-CD	20 mM borate–phosphate pH 7 and 10 mM C.S.	[121]
Fadrozole	CD-MEKC	β-CD, di-OMe-β-CD	20 mM NaH_2PO_4–20 mM sodium tetraborate pH 7, 100 mM SDS, 2 M urea, 15% MeOH and 30 mM C.S.	[88]
Fenfluramine	CZE	di-OMe-β-CD	100 mM phosphoric acid–triethanolamine pH 3, 30% MeOH and 15 mM C.S.	[98]
meta-Fenfluramine	CZE	tri-OMe-β-CD	100 mM phosphoric acid–Tris pH 2.5 and 40 mM C.S.	[105]
orto-Fenfluramine	CZE	γ-CD	100 mM phosphoric acid–Tris pH 2.5 and 30 mM C.S.	[105]
Fenoldopam and trimethyl derivatives	CD-MEKC	β-CD-TDOC	30 mM NaH_2PO_4–10 mM H_3BO_3 pH 7.2 and 20 mM β-CD+50 mM TDOC	[91]
Fenoprofen	CZE	β-CD	200 mM MES pH 4.5, 0.2% HEC and 15 mM C.S.	[78]

(Continued on p. 90)

Table 2 (continued)

Compounds	CE type	CD	BGE	References
	CZE	HP-β-CD	200 m*M* MES pH 4.41, 0.2% HEC and 20 m*M* C.S.	[80]
	CZE	β-CD	600 m*M* MES pH 4.65, 0.2% HEC and 15 m*M* C.S.	[122]
	CZE	tri-OMe-β-CD	100 m*M* MES pH 5 and 30 m*M* C.S.	[62]
Fluoxetine	CD-MEKC	tri-OMe-β-CD	18 m*M* Tris pH 2.7, 0.1% MHEC, CTAB and 10 m*M* C.S.	[106]
Flurbiprofen	CZE	tri-OMe-β-CD	100 m*M* MES pH 5 and 30 m*M* C.S.	[62]
Gallopamil	CZE	HP-β-CD	100 m*M* phosphoric acid–triethanolamine pH 3 and 20 m*M* C.S.	[98]
Glutethimide	CZE	di-OMe-β-CD	50 m*M* phosphate pH 2.5 and 15 m*M* C.S.	[88]
Hexobarbital	CZE	α-CD, β-CD, di-OMe-β-CD	50 m*M* phosphate–10 m*M* borate pH 9 and 10 m*M* C.S.	[123]
	CZE	β-CD, di-OMe-β-CD, HP-β-CD	100 m*M* Tris–boric acid pH 8.3, 2 m*M* EDTA 0.5–1.3% C.S.	[114]
	CD-MEKC	β-CD, di-OMe-β-CD	20 m*M* NaH_2PO_4–20 m*M* sodium tetraborate pH 7, 100 m*M* SDS, 2 M urea, 15% MeOH and 30 m*M* C.S.	[88]
Homatropine	CZE	β-CD	35 m*M* phosphate buffer pH 6.25, 0.2% HEC and 15 m*M* C.S.	[124]
Ibuprofen	CZE	β-CD	200 m*M* MES pH 4.5, 0.2% HEC and 15 m*M* C.S.	[78,125]
	CZE	tri-OMe-β-CD	100 m*M* MES pH 5 and 30 m*M* C.S.	[62]
Imafen	CZE	HP-β-CD	50 m*M* phosphate pH 3.3 and 30 m*M* C.S.	[72]
Imidazole derivatives (enilconazole, ketoconazole, lofexidine, metomidate)	CZE	β-CD or γ-CD	50 m*M* phosphate buffer pH 3, 10% MeOH and 20 m*M* β- or 50 m*M* γ-CD	[75]
imidazole derivatives (econazole, enilconazole, lofexidine, metomidate, miconazole)	CZE	HP-β-CD	50 m*M* phosphate buffer pH 3, 10% MeOH and 20 m*M* C.S.	[75]
Indoprofen	CZE	tri-OMe-β-CD	100 m*M* MES pH 5 and 30 m*M* C.S.	[62]
Isolysergic acid	CZE	γ-CD	0.1 *M* phosphate buffer pH 2.5 and 30 m*M* C.S.	[58]
Isoprenaline	CZE	di-O-Me-β-CD or HP-β-CD	100 m*M* phosphoric acid–triethanolamine pH 3 and 15 or 30 m*M* C.S.	[98]
Isoproterenol	CZE	di-OMe-β-CD	10 m*M* Tris–phosphate pH 2.4 and 18 m*M* C.S.	[14]
	CZE	di-OMe-β-CD	50 m*M* phosphate pH 2.5 and 20 m*M* C.S.	[63]
	CZE	β-CD	150 m*M* phosphate–TBA pH 2.5, and 20 m*M* C.S.	[115]
	CZE	di-OMe-β-CD	50 m*M* sodium phosphate pH 2.5, 70 m*M* TMA and 20 m*M* C.S.	[97]
	CZE	di-OMe-β-CD	50 m*M* phosphate pH 3.3 and 30 m*M* C.S.	[72]
	CZE	β-CD, di-OMe-β-CD, tri-OMe-β-CD, HP-β-CD	50 m*M* TMA–phosphate pH 2.5 and 20 m*M* C.S.	[66]

	CZE	β-CD polymer	50 mM phosphate pH 2.5 and 50 mM C.S.	[69]
Ketamine	CZE	di-OMe-β-CD	50 mM phosphate pH 2.5 and 20 mM C.S.	[63]
	CZE	HP-β-CD	50 mM phosphate pH 3.3 and 30 mM C.S.	[72]
	CZE	β-CD polymer	50 mM phosphate pH 2.5 and 200 mg/ml C.S.	[69]
Ketoprofen	CZE	tri-OMe-β-CD	100 mM MES pH 5 and 30 mM C.S.	[62]
Ketotifen and its intermediates	ITP	β-CD/di-OMe-β-CD	LE, 5 mM sodium acetate pH 5.5, 0.2% HEC and 1 mM C.S.; TE, 10 mM β-alanine	[48]
	ITP/CZE	β-CD	20 mM Tris–citric acid pH 3.5, 0.005% HEC and 10 mM C.S.	[50]
Lisuride	CZE	γ-CD	0.1 M phosphate buffer pH 2.5 and 30 mM C.S.	[58]
Lofexidine	CZE	HP-β-CD	50 mM phosphate pH 3.3 and 30 mM C.S.	[72]
Mefloquine	CZE	HP-β-CD	50 mM phosphate pH 3.3 and 30 mM C.S.	[72]
Meluol	CZE	γ-CD	0.1 M phosphate buffer pH 2.5 and 30 mM C.S.	[58]
Mephenytoin, 4-hydroxymephenytoin	CD-MEKC	β-CD	30 mM NaH_2PO_4–10 mM boric acid pH 7.2, 50 mM TDOC and 20 mM C.S.	[91]
		β-CD	10 mM phosphate–6 mM borate pH 9.1, 100 mM SDS, 10% 2-propanol and 50 mM C.S.	[89]
Mephobarbital	CD-MEKC	β-CD	20 mM NaH_2PO_4–20 mM sodium tetraborate pH 7, 100 mM SDS, 2 M urea, 15% MeOH and 30 mM C.S.	[88]
Mepivacaine	CD-MEKC	di-OMe-β-CD	18 mM Tris–H_3PO_4 pH 2.9, 0.1% MHEC, 0.03 mM HTAB and 10 mM C.S.	[106]
Methomidate	CZE	HP-β-CD	50 mM phosphate pH 3.3 and 30 mM C.S.	[72]
Metoprolol	CZE	di-OMe-β-CD	40 mM Li phosphate pH 3 and 37 mM C.S.	[77]
Merucathinone	CZE	di-OMe-β-CD	90% (25 mM Tris–H_3PO_4 pH 2.45 and 5 mM C.S.), 10% MeOH	[99]
Methamphetamine	CZE	di-OMe-β-CD	98.8% (25 mM Tris–H_3PO_4 pH 2.45 and 5 mM C.S.), 1.2% MeOH	[99]
Metoprolol	CZE	di-OMe-β-CD	50 mM TMA–phosphate pH 2.5 and 20 mM C.S.	[66]
Mianserine	CZE	HP-β-CD	50 mM phosphate pH 3.3 and 30 mM C.S.	[72]
N-methylephedrine	CZE	di-OMe-β-CD	50 mM phosphate pH 3.3 and 30 mM C.S.	[72]
	CZE	di-OMe-β-CD	30 mM Tris–phosphate pH 2.5 and 40 g/l C.S.	[116]
	CZE	di-OMe-β-CD	pH 2.5, HPC, TBA	[117]
	CZE	di-OMe-β-CD	20 mM phosphate pH 2.5, 10 mM TBAB and 18 mM C.S.	[117]
N-methylpseudoephedrine	CZE	di-OMe-β-CD	20 mM phosphate pH 2.5, 10 mM TBAB and 18 mM C.S., HTC, TBA	[117]
	CZE	di-OMe-β-CD	20 mM phosphate pH 2.5, 10 mM TBAB and 18 mM C.S.	[117]

(Continued on p. 92)

Table 2 (continued)

Compounds	CE type	CD	BGE	References
Nadolol	CZE	HP-β-CD	50 m*M* sodium phosphate pH 2.5, 70 m*M* TMA and 20 m*M* C.S.	[97]
	CZE	HP-β-CD	50 m*M* TMA–phosphate pH 2.5 and 20 m*M* C.S.	[66]
Naproxen	CZE	HP-β-CD	200 m*M* MES–TBA pH 5, 0.4% polymeric additive and 10 m*M* C.S.	[126]
	CZE	HP-β-CD	200 m*M* MES pH 4.86, 0.2% HEC and 5 m*M* C.S.	[80]
Nefopam	CZE	HP-β-CD	50 m*M* phosphate pH 3.3 and 30 m*M* C.S.	[72]
Nicergoline	CZE	γ-CD	0.1 *M* phosphate buffer pH 2.5 and 30 m*M* C.S.	[58]
Nomifensine	CZE	HP-β-CD	50 m*M* phosphate pH 3.3 and 30 m*M* C.S.	[72]
Norephedrine	CZE	di-OMe-β-CD	10 m*M* Tris–phosphate pH 2.4 and 20 m*M* C.S.	[14]
	CZE	di-OMe-β-CD	50 m*M* phosphate pH 3.3 and 30 m*M* C.S.	[72]
	CZE	di-OMe-β-CD	30 m*M* Tris–phosphate pH 2.5 and 40 g/l C.S.	[116]
	CZE	di-OMe-β-CD	20 m*M* phosphate pH 2.5, 10 m*M* TBAB and 18 m*M* C.S,	[117]
	CZE	di-OMe-β-CD	90% (25 m*M* Tris–H_3PO_4 pH 2.45 and 5 m*M* C.S.), 10% MeOH	[99]
	CZE	di-OMe-β-CD	50 m*M* TMA–phosphate pH 2.5 and 20 m*M* C.S.	[66]
Norepinephrine	CZE	di-OMe-β-CD	10 m*M* Tris–phosphate pH 2.4 and 18 m*M* C.S.	[14]
	CZE	di-OMe-β-CD	50 m*M* phosphate pH 2.5 and 20 m*M* C.S.	[63]
	CZE	β-CD	150 m*M* phosphate–TBA pH 2.5 and 20 m*M* C.S.	[115]
	CZE	HP-β-CD	30 m*M* Tris–citric acid pH 2.5, 5 m*M* 18C6H_4 and 20 m*M* C.S.	[127,128]
	CZE	di-OMe-β-CD, HP-β-CD	50 m*M* TMA–phosphate pH 2.5 and 20 m*M* C.S.	[66]
	CZE	β-CD polymer	50 m*M* phosphate pH 2.5 and 100 mg/ml C.S.	[69]
Norfenefrine	CZE	HP-β-CD	50 m*M* phosphate pH 3.3 and 30 m*M* C.S.	[72]
Norpseudoephedrine	ITP	β-CD	LE, 5 m*M* sodium acetate pH 5.48 and 10 m*M* C.S.; TE, 10 m*M* β-alanine	[47]
	CZE	di-OMe-β-CD	90% (25 m*M* Tris/H_3PO_4 pH 2.45 and 5 m*M* C.S.), 10% MeOH	[99]
Norverapamil	CZE	tri-OMe-β-CD	100 m*M* phosphoric acid–triethanolamine pH 3 and 25 m*M* C.S.	[98]
	CZE	tri-OMe-β-CD	60 m*M* phosphate pH 2.5 and 60 m*M* C.S.	[129]

O-acetylpseudoephedrine	ITP	β-CD	LE, 5 mM sodium acetate pH 5.48 and 10 mM C.S.; TE, 10 mM β-alanine	[47]
Octopamine	CZE	di-OMe-β-CD	50 mM phosphate buffer pH 2.5 and 20 mM C.S.	[63]
	CZE	HP-β-CD	50 mM phosphate pH 3.3 and 30 mM C.S.	[72]
Oxaminiquine	CZE	β-CD	50 mM NaH_2PO_4 pH 12 and 25 mM C.S.	[93]
Oxomemazine	CZE	HP-β-CD	100 mM phosphoric acid–triethanolamine pH 3 and 15 mM C.S.	[98]
Oxprenolol	CZE	di-OMe-β-CD	50 mM sodium phosphate pH 2.5, 70 mM TMA and 20 mM C.S.	[97]
	CZE	di-OMe-β-CD	50 mM Li phosphate pH 3 and 37 mM C.S.	[77]
	CZE	di-OMe-β-CD, HP-β-CD	50 mM TMA–phosphate pH 2.5 and 20 mM C.S.	[66]
	CZE	β-CD polymer	50 mM phosphate pH 2.5 and 100 mg/ml C.S.	[69]
Pentobarbital	CZE	tri-OMe-β-CD, tri-OMe-α-CD	50 mM phosphate, 10 mM borate pH 9 and 10 mM C.S.	[123]
	CD-MEKC	γ-CD	20 mM phosphate–borate pH 9, 50 mM SDS and 30 mM C.S., 60 mM l-men or 40 mM d-cam	[84]
p-Hydroxynorpseudoephedrine	ITP	β-CD	LE, 5 mM sodium acetate, 0.2% HEC pH 5.48 and 15 mM C.S.; TE, 10 mM β-alanine	[47]
Phenylamines (methyldimethoxy ethyl amphetamine, methyldimethoxy methyla amphetamine, methyl amphetamine, amphetamine, ephedrine, epinephrine, pseudoephedrine)	CZE	HP-β-CD	100 mM citric acid–19.27 mM Na_2HPO_4 pH 2.5 and 120 mM C.S.	[102]
Phenylamines (methyldimethoxymethyl amphetamine, methylamphetamine, methyldimethoxyethylamphetamine,)	CD-MEKC	HP-β-CD	50 mM borate pH 9.5, 5% propan-1-ol, 50 mM STDC and 60 or 120 mM C.S.	[90]
Pholedrine	CZE	HP-β-CD	50 mM phosphate pH 3.3 and 30 mM C.S.	[72]
Picumeterol	CZE	β-CD	200 mM Na_2HPO_4–100 mM citric acid pH 4 and 16 mM C.S.	[15]
	CZE	di-OMe-β-CD	25 mM borate–phosphoric acid pH 2.3 and 30 mM C.S.	[130,131]
Pindolol	CZE	di-OMe-β-CD	50 mM sodium phosphate pH 2.5, 70 mM TMA and 20 mM C.S.	[97]
	CD-MEKC	di-OMe-β-CD	18 mM Tris–H_3PO_4 pH 2.9, 0.1% MHEC, 0.03 mM HTAB and 10 mM C.S.	[106]
	CZE	di-OMe-β-CD	100 mM phosphoric acid–triethanolamine pH 3 and 15 mM C.S.	[98]
	CZE	β-CD, di-OMe-β-CD, HP-β-CD	50 mM TMA/phosphate pH 2.5 and 20 mM C.S.	[66]
Practolol	CZE	di-OMe-β-CD	50 mM phosphate pH 2.5 and 40 mM C.S.	[64]

(Continued on p. 94)

Table 2 (continued)

Compounds	CE type	CD	BGE	References
Primaquine	CZE	di-OMe-β-CD	25 m*M* phosphate, 2 *M* urea, pH 2.5 and 20–40 m*M* C.S.	[111]
	CZE	TM-β-CD	25 m*M* phosphate pH 2.7, 2 *M* urea and 20 m*M* C.S.	[112]
	CZE	γ-CD	25 m*M* phosphate–borate pH 2.7 and 20 m*M* C.S.	[71]
Promethazine	ITP	β-CD	LE: 10 m*M* sodium acetate pH 5.55 and 3 m*M* C.S.; TE: 10 m*M* β-alanine	[132]
	CZE	γ-CD	pH 3	[111]
Propranolol	CZE	β-CD	0.1 *M* phosphate buffer pH 2.5, urea, MeOH and 40 m*M* C.S.	[59]
	CZE	HE-β-CD or HP-β-CD	20 m*M* Tris phosphate pH 2.4 and 28 m*M* C.S.	[96]
	CZE	di-OMe-β-CD	40 m*M* Li phosphate pH 3, MeOH or ACN and 37 m*M* C.S.	[77,133]
Propranolol	CZE	β-CD, di-OMe-β-CD, tri-OMe-β-CD	50 or 100 m*M* phosphate buffer pH 2.5, TBA or TMA and 20 m*M* C.S.	[97,115]
	CD-MEKC	β-CD	40 m*M* borate pH 9.3, 32 m*M* SDS and 12 m*M* C.S.	[100]
	CZE	tri-OMe-β-CD	100 m*M* phosphoric acid–triethanolamine pH 3 and 15 m*M* C.S.	[98]
	CZE	HP-β-CD	200 m*M* TAPSO–TBA hydroxide pH 7, 0.4% polymer additive and 10 m*M* C.S.	[134]
	CZE	HP-β-CD	100 m*M* TAPS pH 7.6 and 15 m*M* C.S.	[101]
	CZE	β-CD, di-OMe-β-CD, tri-OMe-β-CD, HP-β-CD	50 m*M* TMA–phosphate pH 2.5 and 20 m*M* C.S.	[66]
	CZE	β-CD polymer	50 m*M* phosphate pH 2.5 and 20 m*M* C.S.	[69]
Propranolol and derivatives	CZE	HE-β-CD, di-OMe-β-CD, 2,3-di-O-diacetyl-β-CD	50 m*M* KH_2PO_4 pH 3, MeOH and 25 m*M* C.S.	[118]
Pseudoephedrine	ITP	di-OMe-β-CD	LE, 5 m*M* sodium acetate pH 5.48, 0.2% HEC and 10 m*M* C.S.; TE, 10 m*M* β-alanine	[47]
	CZE	β-CD	150 m*M* TBA–phosphate pH 2.5 and 20 m*M* C.S.	[115]
	CZE	di-OMe-β-CD	20 m*M* phosphate pH 2.5, 10 m*M* TBAB and 18 m*M* C.S.	[117]
	CZE	di-OMe-β-CD	50 m*M* TMA–phosphate pH 2.5 and 20 m*M* C.S.	[66]
Pseudomerucathine	CZE	di-OMe-β-CD	90% (25 m*M* Tris–H_3PO_4 pH 2.45 and 5 m*M* C.S.), 10% MeOH	[99]
Quinagolide	CZE	β-CD	50 m*M* phosphate pH 2.5 and 30 m*M* C.S.	[135]
	CZE	α-CD/$18C6H_4$	10 m*M* Tris–citric acid pH 2.2, 20 m*M* $18C6H_4$ and 20 m*M* C.S.	[127]

Salbutamol	CZE	di-OMe-β-CD	pH 3.3	[72]
	CZE	HP-β-CD	phosphate pH 3 (I=0.05), 20 mM TBA and 60 mM C.S.	[67]
Salbutamol and related impurities	CZE	di-OMe-β-CD	50 mM NaH_2PO_4–100 mM citric acid pH 2.5 and 112 mM C.S.	[136]
SDZ EAA 494 (neuroactive drug)	CZE	γ-CD	100 mM borate buffer pH 11 and 20 mM C.S.	[137]
Secobarbital	CZE	α-CD, tri-OMe-α-CD	50 mM phosphate, 10 mM borate pH 9 and 10 mM C.S.	[123]
	CD-MEKC	γ-CD	20 mM NaH_2PO_4–20 mM sodium tetraborate pH 7, 100 mM SDS, 2 M urea, 15% MeOH and 30 mM C.S.	[88]
Serotonin (5-HT) agonist 3-aminoalkyl-6-carboxamido-1,2,3,4-tetrahydrocarbazole and related N-alkyl analogues (GITC)	CD-MEKC	β-CD, STDC	30 mM NaH_2PO_4, 10 mM boric acid pH 7.02 and 20 mM CD, 50 mM STDC	[138]
Sotalol	CZE	HP-β-CD	50 mM phosphate pH 3.3 and 30 mM C.S.	[72]
	CZE	di-OMe-β-CD	100 mM phosphoric acid–triethanolamine pH 3 and 15 mM C.S.	[98]
Suprofen	CZE	tri-OMe-β-CD	100 mM MES pH 5 and 30 mM C.S.	[62]
Synephrine	CZE	di-OMe-β-CD	50 mM phosphate pH 3.3 and 30 mM C.S.	[72]
Terbutaline	CZE	β-CD/di-OMe-β-CD	100 mM phosphate buffer pH 2.5 and 15 mM or 5 mM of C.S.	[59]
	CZE	HP-β-CD or β-CD	100 mM phosphoric acid–triethanolamine pH 3 and 15 mM C.S.	[98]
	CZE	HP-β-CD	phosphate pH 3 (I=0.05), 20 mM TBA and 60 mM C.S.	[67]
	CZE	β-CD polymer	50 mM phosphate pH 2.5 and 10 mg/ml C.S.	[69]
	CZE	β-CD, di-OMe-β-CD, HP-β-CD	50 mM phosphate pH 2.5, 10.6 and 11.6 and 5–25 mM C.S.	[55]
Terguride	CZE	γ-CD	0.1 M phosphate buffer pH 2.5 and 30 mM C.S.	[58]
Thiopental	CD-MEKC	γ-CD	20 mM phosphate–borate pH 9, 50 mM SDS, 60 mM l-men or 40 mM d-cam and 30 mM C.S.	[84]
Thioridazine	ITP	γ-CD	LE: 10 mM sodium acetate pH 5.47, 0.08% HEC and 5 mM C.S.; TE: 10 mM β-alanine	[50]
	ITP	γ-CD	LE: 10 mM NaOH, MES, 0.04% HEC, pH 5.57 and 5 mM C.S.; TE: 10 mM aminocaproic acid	[132]
	CZE	γ-CD	20 mM Tris–H_3PO_4 pH 2.5 and 5 mM C.S:	[50]
Timepidium	CZE	γ-CD	25 mM phosphate–borate pH 2.7 and 20 mM C.S.	[71]
	CD-MEKC	γ-CD	25 mM phosphate–borate buffer pH 9, 50 mM DTAC and 20 mM C.S.	[71]

(Continued on p. 96)

Table 2 (continued)

Compounds	CE type	CD	BGE	References
Tocainide analogues	CZE	γ-CD	40 m*M* sodium phosphate pH 3, 0.05% PVA and 50 m*M* C.S.	[65]
Trimetoquinol	CZE	di-OMe-β-CD,	25 m*M* phosphate, 2 *M* urea pH 2.5 and 10–40 m*M* C.S.	[111]
	CZE	di-OMe-β-CD	25 m*M* phosphate pH 2.7 and 5% C.S.	[71]
Trimetoquinol, analogues	CZE	β-CD polymer	25 m*M* phosphate pH 2.7, 2 *M* urea and 1% C.S.	[112]
	CZE	β-CD or β-CD polymer	25 m*M* phosphate pH 6.5 (2 *M* urea and 40 m*M* β-CD) or 3% β-CD polymer 2.7, 6.5	[71]
Trimipramine	CZE	HP-β-CD	50 m*M* TMA–phosphate pH 2.5 and 20 m*M* C.S.	[66]
Uniconazole	CD-MEKC	γ-CD	100 m*M* borate pH 9, 100 m*M* SDS, 2 *M* urea 10% isobutanol and 50 m*M* C.S.	[56]
Verapamil	CD-MEKC	tri-OMe-β-CD	20 m*M* Tris phosphate pH 2.7, 0.1% MHEC, 0.05 m*M* HTAB, 2% ethylene glycol and 12 m*M* C.S.	[106]
	CZE	tri-OMe-β-CD or β-CD	100 m*M* phosphoric acid–diethanolamine pH 3 and 25 or 15 m*M* C.S.	[98]
	CZE	tri-OMe-β-CD	60 m*M* phosphate pH 2.5 and 60 m*M* C.S.	[129]
	CZE	tri-OMe-β-CD, HP-β-CD	50 m*M* TMA–phosphate pH 2.5 and 20 m*M* C.S.	[66]
Warfarin	CZE	di-OMe-β-CD	100 m*M* phosphate pH 8.35, 2% MeOH and 4 m*M* C.S.	[139]
Zoplicone	CZE	HP-β-CD	50 m*M* phosphate pH 3.3 and 30 m*M* C.S.	[72]

detection provided a 1000-fold improvement in signal-to-noise ratio compared to the UV detection [95]. To our knowledge, this is the first example on using CE-MS for the analysis of enantiomers of pharmaceutical interest and we are convinced that this is an interesting way to identify chiral drug isomers.

The enantiomer separations performed by CE using uncharged CDs as chiral selector included a large number of drugs (antidepressants, antipsychotics, hypnotics, barbiturates, anaesthetics, adrenergic, bronchodilators, non-steroidal anti-inflammatories, anti-asthmatics, anti-histaminics, anticoagulants, anti-fungal drugs, β-blockers, calcium-channel blockers, anti-hypertension drugs, anti-arrhythmics, anti-cholinergics, anti-cancer drugs, anti-malaria drugs, anti-bacterial drugs and aromatase inhibitor) are listed in Table 2.

Among the large family of CDs, those possessing charged/chargeable groups proved to be very effective for the enantiomer separation of a wide range of analytes, including compounds of pharmaceutical interest.

The modification of native CDs by introducing chargeable groups, e.g., methylamino, sulfate, carboxylate, sulfobutyl, allows the use of a charged/chargeable chiral selector with different properties than the parent CD. The presence of the above-mentioned substituent groups increases the solubility of the CD, allows the analysis of uncharged analytes and introduce other interactions, e.g., electrostatic. The modified CDs, under the influence of the applied electric field, move with their own mobility, and the charge of the chiral selector can play a very important role in the separation mechanism.

Considering the simplest theoretical approach described by Wren's group [76,77,140] for enantiomer separation using CDs as chiral selectors, the difference in mobility of the two enantiomers is influenced by the difference in mobility of free analyte (μ_f) and complexed compound (μ_c). The use of charged CDs is causing the increase of these parameters and, thus, the increase of resolution.

Mono(6-β-aminoethylamino-6-deoxy)-β-CD (C-Den) and 2-carboxymethyl-β-CD have been employed in CE for the first time by Terabe and co-workers [13,141] for the enantiomer separation of several dansyl-amino acids, and later on Nardi et al. used positively charged β-CD derivatives, namely 6^A-methylamino-, and 6^A- and 6^D-dimethylamino-β-CD for the enantiomer separation of some hydroxy acid derivatives. Also, several groups investigated different modified CD with charged/chargeable groups for the chiral CE separation of pharmaceutical compounds [16,75,99,102,114,117,142–147].

In a very interesting study Schmitt and Engelhardt used carboxymethylated-β-CD, focusing on the importance of the CD charge on the chiral recognition, which can strongly be influenced by the pH of the BGE. The carboxylic group of the CD was not charged at pH lower than 4 and thus the chiral selector behaved as a quasi stationary phase, while at higher pH (>5) it had its own mobility allowing the chiral separation of uncharged analytes of pharmaceutical interest, such as hexobarbital and oxazolidinone [16]

Additional information on the use of charged CDs has been obtained and discussed by our group using a negatively charged β-CD polymer [143]. The chiral selector possesses very high solubility and can be used either in a charged or uncharged mode selecting the appropriate pH of the BGE. The polymer was successful used for the enantiomer separation of basic compounds of pharmaceutical interest such as terbutaline, propranolol, epinephrine, norephedrine and norphenylephrine. Terbutaline and propranolol seemed to show the highest complexation with the CD polymer that at pH>4 caused an inversion of mobility of the two analytes. The mentioned effect can be used advantageously for reversing the migration order of two enantiomers and thus improve the quantitation when the minor component is migrating behind the major one. To illustrate this Fig. 5 shows the inversion of the migration order of propranolol.

Recently a new charged β-CD derivative (4-sulfobutyl-β-CD, SBE-β-CD) has been synthesised and widely studied in CE for chiral separations. Fig. 6 shows the chemical structure of the SBE-β-CD. The new CD possesses 4 sulfonic groups bound at position 6 of 4 glucopyranose of the CD through the butyl chain. The chiral selector is negatively charged at any pH and thus this feature broadens its use in CE. CE analysis of SBE-β-CD using indirect UV detection revealed that the chiral selector is an heterogeneous mixture [148].

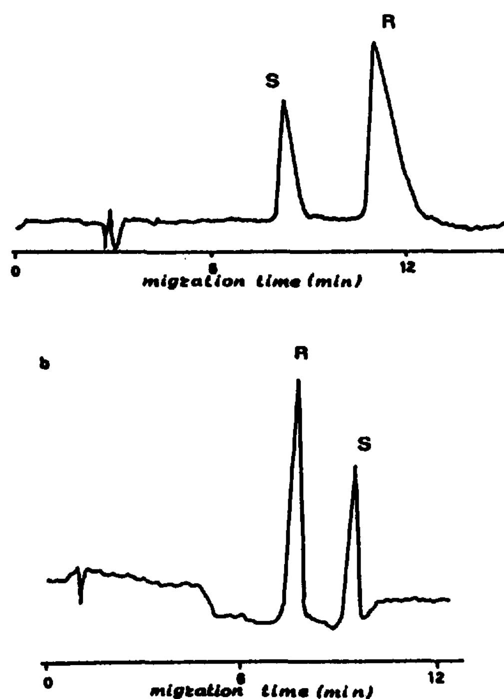


Fig. 5. Inversion of migration order of the two separated enantiomers of propranolol. Capillary 17 cm × 25 μm I.D. (coated); background electrolyte, 50 mM acetate buffer pH 4.5 + (a) 10 mg/ml of negative chiral polymer; (b) reversed polarity (anode direction), 20 mg/ml of negative chiral polymer; applied voltage 12 kV (reprinted with permission from Ref. [143]).

The applicability of SBE-β-CD in CE for the enantiomer separation of pharmaceutical compounds was shown by Dette et al. [117] for the separation of some ephedrine alkaloids (ephedrine, norephedrine, methylephedrine, pseudoephedrine and methylpseudoephedrine) using a BGE at pH 10, close to the pK_a of the studied compounds. The same chiral selector (at a concentration range 1.5–5 mM) was also used by Tait et al. [145] as a chiral selector at pH 2.5 for the enantiomer separation of ephedrine, pseudoephedrine, adrenaline, noradrenaline and DOPA.

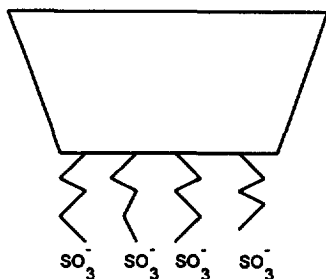


Fig. 6. Structure of the SBE-β-CD.

Basic pharmaceutical compounds (clenbuterol, dimethindene, etilefrine, imafen, isoprenaline, lofexidine, mefloquine, metomidate and mianserine) were resolved in their enantiomers at pH 3.1 using concentrations of SBE-β-CD as low as <40 μM. The chiral selector was also tested in a counter-current flow where the chiral selector was injected for only 1.8 s (high pressure) on cathode as pre-rinse while the analyte (dimethindene) was injected on the anode. Furthermore the negatively charged CD was successfully used for the enantiomer separation of thalidomide, a neutral drug moving with the eof and interacting with SBE-β-CD.

A sulfated-β-CD has been tested as chiral selector for the enantiomer separation of uncharged compounds, such as phensuximide and indapamine, using a BGE at pH 6–8 [149].

Recently we have investigated the effect of several parameters such as CD concentration, pH of the BGE, structure of the analytes on the enantiomer separation of several basic and acidic compounds of pharmaceutical interest using SBE-β-CD [147]. Racemic warfarin, acenocoumarol, terbutaline, bupivacaine, promethazine were successfully separated in their enantiomers. To illustrate this Fig. 7 shows the enantiomer separation of racemic compounds of pharmaceutical interest employing SBE-β-CD as chiral selector.

Table 3 summarizes the isomer separation of chiral drugs by CE using charged CDs as chiral selectors.

3.1.2. Macrocyclic antibiotics

The use of the macrocyclic antibiotics vancomycin, rifamycin B and ristocetin A was recently introduced for chiral separations in CE by Armstrong et al. [150–152]. Table 4a and b show the compounds of pharmaceutical interest resolved in their enantiomers using macrocyclic antibiotics as chiral selector.

Different basic compounds of pharmaceutical interest including adrenergic, vasocontrictors, bronchodilators, vasodilators, β-adrenergic blockers were

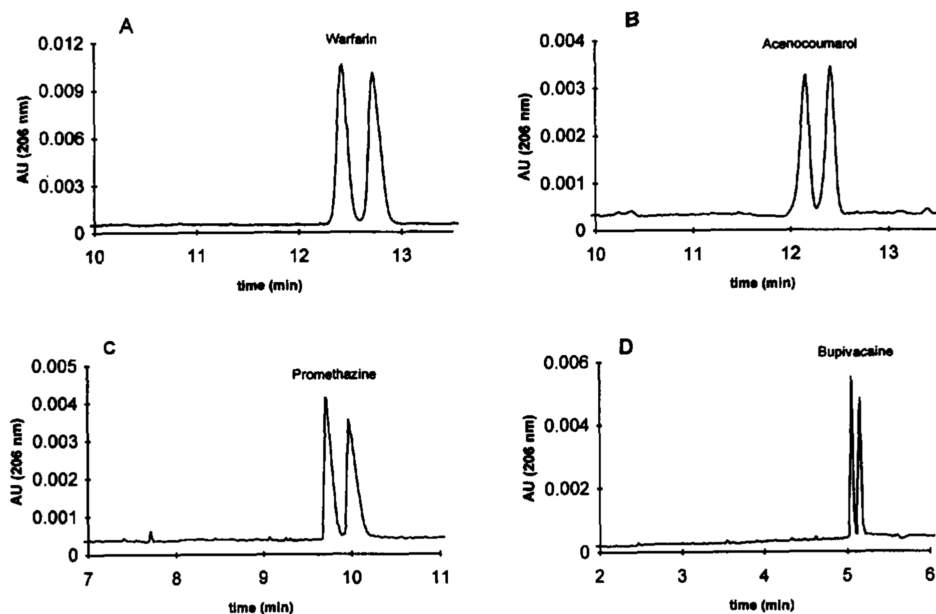


Fig. 7. Electropherograms of the enantiomer separation of racemic compounds of pharmaceutical interest when a sulfobutylated-β-CD is used as chiral selector. Background electrolyte 50 m*M* phosphate buffer pH 6 containing SBE-β-CD (A, B and C, 6 mg/ml; D, 10 mg/ml); capillary 50(45.5) cm × 50 μm I.D.; applied voltage 15 kV (reprinted with permission from Ref. [147]).

resolved in their enantiomers using rifamycin B as chiral selector dissolved in an organic–aqueous buffer due to its low solubility in water. Due to the strong absorption at relatively short UV wavelengths of the chiral compound indirect UV detection was employed. As can be seen in Fig. 8 rifamycin B possesses one carboxylic group that was dissociated at the operating pH (pH 7) and thus the chiral compound was moving as negatively charged in the opposite direction of the analysed basic compounds. Different types of interactions were involved in the chiral recognition process, namely charge-charge, hydrogen-bonding and hydrophobic-inclusion.

Rifamycin B proved to be a good chiral selector for amino alcohols and its chiral enantioselectivity was strongly influenced by the chemical structure of analytes (the position of the hydroxy group, the amine type). In fact, when the OH group was at the position of the aromatic ring or a secondary amino group was present the chiral recognition was enhanced. Furthermore, the presence of more than one aromatic ring in the analyte's structure reduced the possibility of enantiomeric resolution, indicating the importance of the inclusion interaction.

Among other operational parameters the organic modifier type and concentration, the BGE type and its ionic strength, the concentration of rifamycin strongly influenced the chiral recognition of the studied basic compounds. The optimum experimental conditions were found using 0.1 *M* phosphate buffer at pH 7 (60%), 2-propanol (40%) and 25 m*M* of rifamycin B.

Vancomycin and ristocetin are macrocyclic glycopeptide antibiotics that showed no mobility at a pH of about 7; at lower and higher pH they are positively and negatively charged, respectively. The two chiral selectors have been used for the chiral separation of more than 100 compounds, including some of the pharmaceuticals listed in Table 4b. The resolution of naproxen increased by increasing the concentration of vancomycin, while increasing the pH reduced the enantioselectivity [150]. A similar effect was recorded for ketoprofen when ristocetin was studied [153].

In a recent paper SDS was added to the BGE containing vancomycin for the enantiomer separation of several dansyl-amino acids and pharmaceutical compounds. The addition of the surfactant to the

Table 3
Chiral drugs isomer separation by capillary electrophoresis using modified charged CDs

Compound	Resolution	Chiral selector	BGE	Capillary size	References
Doxylamine	1.8	CM-β-CD (2%)	20 m*M* phosphate buffer	37(30) cm × 75 μm I.D.	[16]
Ephedrine	3.5		pH 5.8 and 2% C.S.		
Dimethindene	0.9				
Propranolol	3.6				
Dimethindene	base-line	CM-β-CD (2%)	20 m*M* citric acid pH 2.5	27(20) cm × 75 μm I.D.	[114]
Doxylamine			and 2% C.S.		
Ephedrine					
	base-line	CM-β-CD (2%)	20 m*M* citric acid pH 2.5 and 2% C.S.	67(60) cm × 75 μm I.D.	[146]
Arterenol					
Dimethindene					
Doxilamine					
Ephedrine					
Pindolol					
Propranolol					
Hexobarbital	0.8	CE-β-CD (2%)	20 m*M* phosphate buffer pH 5.8 and 2% C.S.	37(30) cm × 75 μm I.D.	[16]
GR5788X	base-line	CM-β-CD (25 m*M*)	10 m*M* Tris pH 12.4	72 cm × 50 μm I.D.	[142]
Aminoglutethimide	base-line	CM-β-CD (5 m*M*) and β-CD (1 m*M*)	NaH_2PO_4–$Na_2B_4O_7$ pH 9, 50% MeOH	50 cm × 50 μm I.D.	[92]
Imidazole derivatives		SBE-β-CD (0.1 m*M*)	50 m*M* phosphate buffer pH 3, 20% MeOH	61(44) cm × 50 μm I.D.	[75]
Bifonazole	1.01				
Econazole	2.46				
Enilconazole	2.34				
Miconazole	1.44				
Ketoconazole	1.66	SBE-β-CD (0.1 m*M*)	50 m*M* phosphate buffer pH 3, 40% MeOH		
Lofexidine	1.03	SBE-β-CD (0.1 m*M*)	50 m*M* phosphate buffer pH 3		
Metomidate	2.21				
		SBE-β-CD (1 m*M*)	1.2% methanol and 98.8% 25 m*M* Tris–phosphate pH 2.4 and C.S.	82(60) cm × 50 μm I.D.	[99]
Cathinone	1.9				
Norpseudoephedrine	6.8				
Amphetamine	3.0				
Methcatinone	3.6				
Pseudoephedrine	6.2				
Ephedrine	1.5				
Metamphetamine	3.1				
		SBE-β-CD (mg/ml)	50 m*M* phosphate buffer pH 6	50(45.5) × 50 μm I.D.	[147]
Acenocoumarol	1.4	6			
Bupivacaine	1.36	10			
Promethazine	1.28	6			
Terbutaline	1.56	1			

Warfarin	1.35	6			
Atenolol	0.67	SBE-β-CD (2 m*M*)	20 m*M* citric acid–phosphate buffer pH 2.5	100 cm (50) × 50 μm I.D.	[102]
Cimaterol	0.75				
Clenbuterol	1.31				
Salbutamol	1.00				
Terbutaline	2.80				
Thalidomide	2.25				
Amphetamine	1.33	SBE-β-CD (4.6 m*M*)	20 m*M* citric acid–phosphate buffer pH 2.5	100 cm (50) × 50 μm I.D.	[102]
Atenolol	3.13				
Clenbuterol	1.67				
Methylamphetamine	1.78				
Terbutaline	2.88				
Thalidomide	2.00				
Fluoxetine	1.71	SBE-β-CD (7.5 m*M*)	1% triethanolamine–acetic acid pH 5.5 and 10% ACN	57 (50) cm × 50 μm I.D.	[94]
Ephedrine	1.13	SBE-β-CD (40 m*M*)	20 m*M* borate buffer pH 10	50 cm (effective length) × 50 μm I.D.	[117]
Methylephrine	1.27				
Methylpseudoephedrine	3.08				
Nor-ephedrine	0.71				
		sulfated-β-CD(SO_3-β-CD) (2–4%)	10 m*M* Na_2HPO_4	60 (52.4) cm × 75 μm I.D.	[149]
Phensuximide	1.94		pH 7 (3% CD)		
Hydantoin derivatives	2.94–3.95		pH 8, 10% MeOH		
Indapamide	1.50		pH 8 (2% CD)		
		SBE-β-CD (m*M*)	50 m*M* phosphate buffer pH 3.10	41 cm (effective length) × 50 μm I.D.	[144]
Clenbuterol	0.68	1.00			
Dimethindene	1.18	0.08			
Etilephrine	1.43	1.00			
Imafen	4.53	1.00			
Isoprenaline	1.04	1.00			
Lofexidine	3.15	1.00			
Mefloquine	1.51	0.08			
Metomidate	2.21	1.00			
Mianserine	1.17	0.05			

Table 4
Compounds of pharmaceutical interest resolved in their enantiomers using macrocyclic antibiotics as chiral selectors

(a) Enantiomeric separation of basic compounds of pharmaceutical interest using 25 m*M* of rifamycin B by CE using indirect UV detection at 254 nm. Background electrolyte 60% 0.1 *M* phosphate buffer/40% 2-propanol and chiral selector. Capillary: 57.5 (50) cm × 0.05 mm I.D.; applied voltage 8 kV; injection 5 kV, 5 s of 1 mg/ml of racemic analyte [152].

Compound	Resolution	Compound	Resolution
Atenolol	0.7	Metaproterenol	1.8
Alprenolol	0.7	Metoprolol	0.8
Bamethan	1.9	Norepinephrine	0.9
Ephedrine	1.4	Normetanephrine	0.9
Epinephrine	1.5	Norphenylephrine	1.5
ψ-Ephedrine	1.4	Octopamine	1.1
Isoproterenol	2.3	Oxprenolol	0.4
Metanephrine	1.6	Salbutamol	1.5
Synephrine	1.7	Terbutaline	3.1

(b) Enantiomers resolution of pharmaceutical compounds using (a) 2 m*M* of ristocetin, b) 5 m*M* vancomycin. Background electrolyte: 0.1 *M* phosphate buffer at pH a=6, b=7 and chiral selector; Capillary: 32.5 (25) cm × 0.05 mm I.D.; applied voltage, 5 kV, injection: hydrostatic 3–5 s; 22°C [150,153]

Compound	Resolution		Compound	Resolution	
	a	b		a	b
Carprofen	1.2	3.5	Methotrexate	2.3[a]	
Fenoprofen	0.9	3.6	Naproxen	5.8	6.5
Flurbiprofen	1.7	8.2	Proglumide	1.2[b]	
Ibuprofen		4.7	Suprofen	1.4	3.0
Indoprofen	1.3	2.2	Folinic acid[c]	1.3	3.4
Ketoprofen	5.7	4.5			

[a] 2 m*M* vancomycin.
[b] 1 m*M* vancomycin.
[c] Diastereomeric separation.

chiral environment decreased the migration time of analytes, improved the efficiency and reversed the migration order of the studied enantiomers. The pharmaceutical compounds resolved in their enantiomers employing the SDS-vancomycin buffer include flurbiprofen, ketoprofen, carprofen, indoprofen and suprofen [154].

Rifamycin B

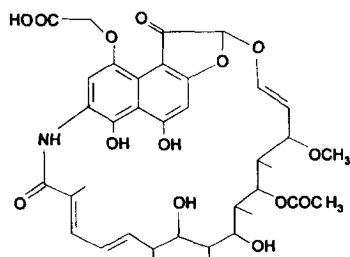


Fig. 8. Structure of rifamycin B.

3.1.3. Crown-ether derivatives

Crown-ethers represent a family of macrocyclic polyethers able to form host-guest complexes with several inorganic and organic cations like alkaline and earth metal ions, as well as organic compounds with amino groups. The use of crown-ethers as complexing agent for the separation of inorganic ions is documented in ITP [155]. The chirality of the crown-ethers can be obtained modifying their chemical structure, e.g., introducing four carboxylic

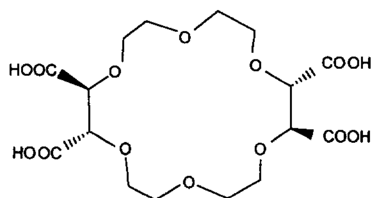


Fig. 9. Structure of 18-Crown-6-ether tetracarboxylic acid.

groups. To illustrate this, Fig. 9 shows the chemical structure of 18-crown-6-ether-tetracarboxylic acid.

The resolution mechanism for enantiomer separation, i.e., inclusion-complexation, is not the same as for CDs. In fact, here the analyte fits in the crown-ether's cavity with its hydrophilic part (protonated amino group) on forming ion–dipole bonds with the oxygen atoms of the chiral selector. The inclusion complex is stabilised by secondary bonds between substituent groups on the asymmetric centre of the analyte and the carboxylic groups of the crown. These latter bonds (hydrogen and/or electrostatic) can explain the chiral separation of amino compounds [156]; however, when non-polar substituents are present in the structure of analytes the enantioselectivity is strongly influenced by the size of the substituent groups (barrier effect).

When modified crown-ether has to be used in CE, the BGE type should be carefully selected avoiding the use of cations that can interact with the chiral selector such as ammonium, sodium or potassium ions.

The chiral selector has been used recently for the enantiomer separation of several amines including compounds of pharmaceutical interest like noradrenaline, norephedrine and normetanephrine [156]. A synergistic effect on the chiral separation of noradrenaline was found combining modified crown-ether and HP-β-CD on forming a ternary complex with the analytes [128].

3.2. Affinity electrophoresis

Among the large number of chiral selectors investigated in CE for the enantiomer separation of pharmaceutical compounds are also proteins. These chiral selectors are natural compounds composed by several amino acids in their polymeric structure, they can be positively or negatively charged changing the pH of the BGE (to values below or above their pI, respectively). Thus changes of the pH could be a very important parameter for enantiomer separation because the number of interaction points in the protein can be modified for an improvement of stereoselectivity. When a protein is charged, it is moving with its own mobility and the analysis of both charged and uncharged analytes can be performed, and thus the protein system is similar to a micellar phase. In this case the interactions involved in the chiral separation mechanism can be either attractive or repulsive.

To date, several proteins have been used in CE for the enantiomer separation of pharmaceutical compounds, including BSA [157–162], α_1-AGP [162], avidin [163], HA [164,165], conalbumine [162], fungal cellulase [157], orosomucoid [157], OVM [162,166] and cellobiohydrolase I [167].

Bovine serum albumin has been widely used in CE for enantiomers resolution of pharmaceutical compounds, e.g., warfarin, using a phosphate buffer at pH 6.8, and the addition of isopropanol to the BGE improved the resolution. In the same study fungal cellulase at pH 7.4 allowed the resolution of pindolol enantiomers, and α_1-glycoprotein resolved the racemic mixture of promethazine [157]. The spatial arrangement of proteins can be influenced by several parameters such as pH, temperature and BGE composition; this has been shown in CE using HSA as chiral selector [164] after heating the protein in weakly alkaline aqueous solution, selectivity and resolution of 2,3-dibenzoyl-DL-tartrate improved.

The separation of optical isomers (diastereoisomers) of leucovorin (6R)- and (6S)-diastereoisomers was obtained by MEKC using bovine serum albumin (BSA) added to the BGE at pH 7 and 7.2. A poly(ethylene glycol) (PEG)-coated capillary was used in order to improve the reproducibility reducing the BSA adsorption and to reverse the migration order. At the pH used both analytes and BSA were negatively charged and the (6R)-isomer had a greater affinity for BSA than the (6S)-isomer. The method, after studying the effect of protein concentration and pH, was mainly used for thermodynamic data measurements [158]. The same chiral selector (BSA) with dextran was employed for the separation of leucovorin diastereoisomers and ibuprofen enantiomers using a relatively short capillary (20 cm) coated

with polyacrylamide [159]. Better results were obtained by Sun et al. [160] for the diastereoisomeric separation of leucovorin using immobilised BSA-dextran polymer network.

Tanaka and Terabe employed a discontinuous separation zone (see also Ref. [167] discussed in this review) filling only a part of the capillary with BGEs containing different proteins in order to avoid detection problems for the enantiomer separation of several pharmaceutical compounds. The electrophoretic method was evaluated for reproducibility (migration times and peak areas) obtaining comparable results with conventional CE; epinastine was used as model and separated by BSA as a chiral additive [162].

Recently it has been shown that heparin has great potential as chiral selector in capillary electrophoresis for the enantiomer separation of several drugs, e.g., antimalarial compounds, antihistamines. Heparin is a polyanionic glycosaminoglycan with a molecular mass between 10 and 30 kDa and thus negatively charged. Good enantiomeric resolution was obtained using a BGE at pH 4.5 or 5 supported with 2% heparin (10 kDa). The analytes were moving toward the cathode as cation and retarded due to the interactions with the chiral selector and the stereoselectivity was due to the combination of ionic, hydrogen and hydrophobic interactions [168].

Nishi et al. [169] employed a charged polymer, dextran sulfate, composed by several glucose units connected to each other though α-(1,6)-bonds for the enantiomers resolution of trimetoquinol and one of its isomer and two natural mucopolysaccharides, namely chondroitin sulfate and heparin were employed for the enantiomer separation of several drugs and the results were compared with those obtained using dextran sulfate. Trimetoquinol, trimetoquinol isomer, norlaudanosoline, laudanosoline, laudanosine, chloropheniramine were separated in their enantiomers with the mucopolysaccharides used and the best results were obtained with chondroitin at acidic pH. Good linearity, reproducibility and recovery was achieved in the analysis of trimetoquinol enantiomers when chondroitin sulfate was investigated as a chiral selector. The optimised method (acidic buffer containing 3% of chiral selector) was applied for the purity test control of drugs. The method allowed to detect less than 0.1% of either *R*-diltiazem and *R*-trimetoquinol [170].

The use of linear saccharides as chiral selectors in CE has been shown by D'Hulst and Verbeke [171]. α-(1,4)-linked D-glucose polymers (maltodextrins) and maltooligosaccharides (corn syrups) were dissolved in a BGE at pH 7 for the enantiomer separation of compounds of pharmaceutical interest such as ibuprofen, flurbiprofen, warfarin, coumarinic derivatives and cefalosporin antibiotic.

Simendan, ibuprofen, warfarin and ketoprofen were separated in their enantiomers using dextrin 10 or Maltrin M040 and the resolution mechanism was discussed also considering the results obtained by ^{1}H- and ^{13}C-NMR studies [172].

Quang and Khaledi studied Dextrin 10, dextrin 15 and dextrin 20 at pH 6–7 for the chiral separation of five non-steroidal anti-inflammatory drugs. Flurbiprofen, suprofen, indoprofen, ibuprofen and fenoprofen were successfully resolved in their enantiomers. Better chiral resolution was obtained using dextrins with the lowest DE and decreasing the column temperature. The chiral separation of racemic warfarin was also obtained with dextrin 10 at pH 5.1 [173].

Recently dodecyl β-D-glucopyranoside monophosphate and -monosulfate have been synthesised by Tickle et al. and successfully employed for the enantiomers resolution of several pharmaceutical compounds such as metoprolol, ephedrine, hexobarbital, phenobarbital and fenoldopam. The new chiral selectors possess relatively low critical micelle concentrations (0.5 and 1 m*M*, respectively) [174].

A charged polymer, dextran sulfate, composed by several glucose units connected to each other though α-(1,6)-bonds was employed by Nishi et al. [169] for the enantiomer resolution of trimetoquinol and one of its isomer. The resolution of the studied enantiomers was strongly influenced by the concentration of the chiral selector and by the pH of the BGE. Using 3% dextran sulfate the resolution was optimal at pH 5.5–8.4 and decreased by increasing the pH. The addition of either urea or organic additive deteriorated the resolution.

Table 5 shows the list of enantiomers of pharmaceutical interest separated by CE using affinity interactions.

Table 5
Pharmaceutical compounds separated in their enantiomers using affinity interactions

Chiral selector	Drug	BGE	Capillary	References
α_1-AGP	Clorprenaline	50 m*M* phosphate buffer pH 4 and 500 μM C.S.	36(31.5) cm × 50 μm I.D. (coated)	[162]
Avidin	Flurbiprofen	50 m*M* phosphate buffer pH 6, 10% ethanol and 25 μM C.S.	36(31.5) cm × 50 μm I.D. (coated)	[163]
	Folinic acid[a] Ibuprofen Ketoprofen			
	Warfarin	50 m*M* phosphate buffer pH 6, 10% THF and 25 μM C.S.	36(31.5) cm × 50 μm I.D. (coated)	[163]
BSA	DR-3862 (antimicrobial agent, e.g., of laxacin)	100 m*M* phosphate buffer pH 8 and 0.4% C.S.	45 cm × 75 μm I.D.	[161]
	Epinastine	50 m*M* phosphate buffer pH 6.0 and 750 μM C.S.	36(31.5) cm × 50 μm I.D. (coated)	[162]
	Homochlorcyclizine	50 m*M* phosphate buffer pH 6 and 500 μM C.S.	36(31.5) cm × 50 μm I.D. (coated)	[162]
	Ibuprofen	10 m*M* phosphate buffer pH 7.12 + 5% dextran and 1 mg/ml BSA	20 cm × 75 μm coated with polyacrylamide	[159]
	Oflaxacin	100 m*M* phosphate buffer pH 8 and 0.4% C.S.	45 cm × 75 μm I.D.	[161]
	Oxyphencyclimine, Propranolol, Trimebutine	50 m*M* phosphate buffer pH 6 and 500 μM C.S.	36(31.5) cm × 50 μm I.D. (coated)	[162]
	Promethazine	50 m*M* phosphate buffer pH 7.4, 3% n-propanol and 50 μM C.S.	50.7 cm × 50 μm I.D.	[157]
	Warfarin	50 m*M* phosphate buffer pH 6.8 and 50 μM C.S.	50.7 cm × 50 μm I.D.	[157]
BSA	Leucovorin[a]	10 m*M* phosphate pH 7.12 + 5% dextran and 1 mg/ml BSA	20 cm × 75 μm coated with polyacrylamide	[159]
BSA	Leucovorin[a]	20 m*M* phosphate buffer pH 7 or 7.2+1 mg/ml BSA	95 cm × 75 μm coated with 20% PEG	[158]
BSA-dextran (polymer network)	Leucovorin[a]	20 m*M* phosphate buffer pH 7.02 (BSA 1 mg/ml)	20 cm × 75 μm coated with polyacrylamide	[160]
Cellobiohydrolase I	Alprenolol, Metoprolol, Propranolol, Pindolol	400 m*M* sodium phosphate pH 5.1, 25% 2 propanol and 40 ng/ml C.S.	11.5(8.5) cm × 75 μm I.D. (coated)	[167]
Chondroitin sulfate	Chlorodiltiazem Diltiazem Trimetoquinol and related compounds	20 m*M* phosphate–borate pH 2.4 and 3% C.S.	57(50) cm × 75 μm I.D.	[170]
Conalbumine	Trimetoquinol	50 m*M* phosphate buffer pH 7 and 500 μM C.S.	36(31.5) cm × 50 μm I.D. (coated)	[162]
Dextran sulfate	Diltiazem Trimetoquinol and its isomers	20 m*M* phosphate/borate pH 5.5 and 3% C.S.	57(50) cm × 75 μm I.D.	[170]
Dextrin	Fenoprofen Ketoprofen Warfarin	100 m*M* phosphate/pyrophosphate pH 6 or 7 and 10% C.S.	62(50) cm × 52 μm I.D.	[173]

Table 5 (*continued*)

Chiral selector	Drug	BGE	Capillary	References
Fungal cellulase	Pindolol	50 m*M* phosphate pH 7.4 and 20 μM C.S.	50.7 cm × 50 μm I.D.	[157]
Glucidex 2	*p*-Chlorophenprocoumon *p*-Chlorowarfarin Phenprocoumon Warfarin	10 m*M* Tris/phosphate pH 7 and 3% C.S.	60 cm × 50 μm I.D.	[175]
HA	Kynunerine	10 m*M* borate buffer pH 9.5 and 1 mg/ml C.S.	70(54) cm × 80 μm I.D.	[164]
Heparin	Arabasine Bromopheniramine Bupivacaine Carbinoxamine Chloroquine Chlorpheniramine Dimethindene Doxylamine Enpiroline Hydrochloroquine Mefloquine Nornicotine Primaquine Pheniramine Quinacrine Tetranisole	10 m*M* Na_2HPO_4/ H_3PO_4 pH 5 and 2% C.S.	60(52.4)cm × 75 μm	[168]
	Chlorodiltiazem Chloropheniramine	20 m*M* phosphate–borate pH 6 and 3% C.S.	57(50) cm × 75 μm I.D.	[170]
	Oxaminiquine	50 m*M* NaH_2PO_4 pH 3 and 3 m*M* C.S.	71(50) cm × 50 μm I.D.	[93]
HSA	Proprionazine Promethazine Thioridazine	50 m*M* phosphate pH 7 and 35 μM C.S.	72(50) cm × 50 μm I.D.	[165]
Maltodextrin	Ibuprofen Ketoprofen Simendan Warfarin	20 m*M* TAPS/6.5 m*M* Tris–4% ethanol pH 7.7 and 15 m*M* Dextrin 10	60(44) cm × 50 μm I.D.	[172]
	Fluoxetine Norverapamil Verapamil	25 m*M* Tris–H_3PO_4 pH 3.4 and 20% Dextrin 10	60(44) cm × 50 μm I.D. (coated)	[172]
Maltooligosaccharides	Flurbiprofen Ibuprofen Ketoprofen	10 m*M* sodium phosphate pH 7 and 2.5–10% C.S.	50 cm × 75 μm I.D. or 90 cm × 75 μm I.D. or 60 cm × 50 μm I.D.	[171]
Orosomucoid	Promethazine	50 m*M* phosphate pH 6.8 and 21 μM C.S.	50.7 cm × 50 μm I.D.	[157]
Ovomucoid (OVM)	Arotinolol	50 m*M* phosphate buffer pH 5, 6% 2-propanol and 500 μM C.S.	36(31.5) cm × 50 μm I.D. (coated)	[162]
	Bunitrolol	50 m*M* phosphate buffer pH 5, 10 m*M* CHAPS and 500 μM C.S.	36(31.5) cm × 50 μm I.D. (coated)	[162]
	Chlorpheniramine	10 m*M* phosphate buffer pH 5, 9% 2-propanol and 250 μM C.S.	57(50) cm × 75 μm I.D.	[166]
	Chlorpheniramine Oxyphencyclimine Primaquine Trimebutine	50 m*M* phosphate buffer pH 5, 8% 1-propanol and 500 μM C.S.	36(31.5) cm × 50 μm I.D. (coated)	[162]

(*Continued on p. 107*)

Table 5 (*continued*)

Chiral selector	Drug	BGE	Capillary	References
	Eperisone	50 m*M* phosphate buffer, 0.5 *M* PEA, pH 5 and 50 μ*M* C.S.	57(50) cm × 75 μm I.D.	[166]
	Pindolol	50 m*M* phosphate buffer pH 5, 8% ethanol and 500 μ*M* C.S.	36(31.5) cm × 50 μm I.D. (coated)	[162]
	Tolperisone	10 m*M* phosphate buffer pH 5.5, 0.1% HPC	57(50) cm × 75 μm I.D.	[166]
	Tolperisone, Verapamil	50 m*M* phosphate buffer pH 5, 10% 2-propanol and 500 μ*M* C.S.	36(31.5) cm × 50 μm I.D. (coated)	[166]

[a] Diastereoisomers.

3.3. Micelles

Micellar phases have been widely used in CE, mainly for the separation of uncharged compounds. The technique, introduced by Terabe, was named micellar electrokinetic chromatography (MEKC) [176]. The separation system is composed by two phases, the aqueous BGE and a charged surfactant forming micelles moving with their own electrophoretic mobility. A strong electro-osmotic flow pushes both neutral analytes and micelles to the detector. The neutral compounds are interacting with the micellar and the aqueous phases and the migration order is a function of the distribution coefficient of the analytes and the two phases.

The applicability of MEKC to the enantiomer analysis employing different kinds of chiral surfactants, like bile salts and N-dodecanoyl-L-valinate has been shown first by Terabe's group [177].

Derivatised optical active amino acids, natural surfactants or achiral surfactants/CDs have been shown to offer several possibilities for the optical isomer separation by MEKC.

The use of chiral derivatised amino acids like sodium dodecanoyl-L-valinate (SDVal) or sodium dodecanoyl-L-alalinate (SDAla) in MEKC is well documented for the enantiomer separation of derivatised amino acids [178–181].

The applicability of SDVal to the enantiomer separation of pharmaceutical compounds like warfarin has been shown using a mixed micelle system SDVal and SDS at pH 9 in the presence of 10% methanol and 5 *M* urea [182].

Recently a novel chiral surfactant, N-dodecoxycarbonylvaline *S* or *R* configuration, was synthesised and used in MEKC at pH>7 (at lower pH the surfactant was not soluble) for the enantiomer separation of several compounds of pharmaceutical interest. Atenolol, bupivacaine, ephedrine, homatropine, ketamine, metoprolol, N-methylpseudoephedrine, norephedrine, norphenylephrine, octopamine, pindolol and terbutaline were successfully resolved in their enantiomers using a phosphate/borate buffer at pH 8.8 and 25 m*M* of *S*-N-dodecoxycarbonylvaline. Compared to the dodecanoyl-valinate the novel chiral surfactant *S*-N-dodecoxycarbonoylvaline, at the same experimental condition, proved to be a better resolving agent [183].

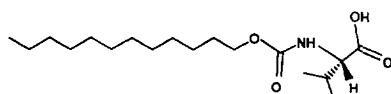
Dodecyl β-D-glucopyranoside monophosphate and monosulfate possessing low CMC (0.5 and 1 m*M*) were synthesised and used as chiral selectors for the enantiomer separation of drug compounds such as mephenytoin and hydroxymephenytoin, metoprolol, ephedrine, hexobarbital, phenobarbital and fenoldopam. The advantages of the new chiral selectors include the low CMC, their high solubility in aqueous media, the low absorbance at short wavelengths and the fact that they can be synthesised in both the L- and D-form [174].

Another very interesting class of chiral surfactants forming micelles is represented by bile salts, they are natural chiral compounds with a helical structure able to interact with relatively flat and rigid compounds where electrostatic, hydrogen and hydrophobic interactions are involved in the separation mechanism [177]. The most commonly used bile salts are sodium cholate (SC), sodium deoxycholate (SDC), sodium taurocholate (STC) and sodium taurodeoxycholate (STDC). SC and SDC dissolve well only in neutral or alkaline solutions while, due

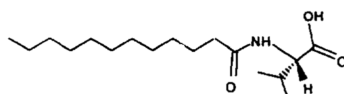
to the presence in their structure of taurine, STC and STDC have a good solubility in water. Fig. 10 shows the chemical structure of some chiral surfactants used for drug enantiomer separations.

Bile salt micelles have been employed by Terabe et al. [177] in MEKC for the chiral resolution of several dansyl-amino acids. Nishi et al. showed the possibility to use bile salts for chiral resolution of drugs under neutral or alkaline solutions. The chiral recognition was affected by the structure of the chiral selector, e.g., trimetoquinol and diltiazem could only be resolved by STDC. The chiral selector increased the solubilization and allowed electrostatic interactions due to the presence of taurine [184]. Nishi et al. [185,186] extended the study to other drug isomers (laudanosoline, norlaudanosoline, laudanosine); diltiazem and trimetoquinol were resolved in their enantiomers at neutral pH and the possibility to detect less than 1% of minor component (*R*-trimetoquinol in the presence of 99% of *S*-trimetoquinol) was shown.

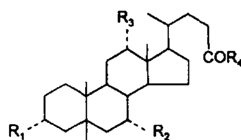
(S)-N-dodecoxycarbonylvaline



(S)-N-dodecanoylvaline



Bile salts



Surfactant Sodium Salts	R1	R2	R3	R4
Cholate (SC)	OH	OH	OH	ONa
Taurocholate (STC)	OH	OH	OH	$NHC_2H_4SO_3Na$
Deoxycholate (SDC)	OH	H	OH	ONa
Taurodeoxycholate (STDC)	OH	H	OH	$NHC_2H_4SO_3Na$

Fig. 10. Structure of some chiral surfactants used in MEKC.

A mixed chiral MEKC phase containing STDC and β-CD was used for the enantiomer separation of standard fenoldopam and one of its derivatives [91]. Microemulsion system composed by a lipophilic chiral compound di-*n*-butyl-tartrate (oil), SDS (surfactant), 1-butanol (cosurfactant) and BGE at pH 8.1 has been used for the chiral separation of ephedrine [187].

Recently two new chiral selectors were employed for the separation of enantiomers by MEKC by El Rassi and Mechref. N,N-bis(3-D-gluconamidopropyl)cholamide (Big CHAP) or deoxycholamide (Deoxy Big CHAP) displayed two chiral portion, namely the steroidal and the polar polyhydroxy moieties. The charge of the chiral micelles was adjusted by changing the pH and the borate concentration. Lowering the capillary temperature and the addition of 10–15% methanol improved the enantiomeric resolution of compounds like dansyl-amino acids and herbicides. Due to their properties the new chiral surfactants could broaden the applications in chiral pharmaceutical analysis [188].

Finally it is worth to mention a very interesting approach in MEKC: the use of chiral micelle polymers, poly(sodium N-undecylenyl-L-valinate) has been shown to be a good chiral selector for (±)1,1′-bi-2-naphtol and D,L-laudanosine. Enhanced stability and rigidity are the main advantages of the polymer in comparison to the monomer. Furthermore, the polymer can be used at any concentration because the polymicelles have no CMC [189].

3.4. Chiral drug analyses by electrochromatography

One interesting way for chiral separation by CE technique is the use of electrochromatography (EC). The chiral selector is bound to the capillary wall or is a chiral stationary phase (CSP) packed capillary. The mobile phase is moved by the electro-osmotic flow with a flat profile, which allows to obtain higher efficiencies than in liquid chromatography where the profile is parabolic. Both systems were successfully applied in the enantiomer separation of compounds of pharmaceutical interest.

A capillary tube of 50 μm I.D. was coated with permethylated β- or γ-CD (Chirasil-Dex) and used

by Mayer and Schurig in EC for the enantiomer separation of several non steroidal antiinflammatory drugs (NSAIDs) ibuprofen, cicloprofen, flurbiprofen and etodolac. The effect of operational parameters, e.g., applied voltage, pH of the buffer, thickness of the coating and electro-osmotic flow on resolution and efficiency were studied. The increase of the film thickness caused a reduction in efficiency due to the low speed of mass transfer [190]. The same group [191] separated hexobarbital enantiomers and investigated the effect of β-CD derivatives (charged and uncharged) using a capillary modified with Chirasil-Dex (Dual chiral recognition system).

Another interesting approach was shown by Szeman and Ganzler [192] using CDs-coated capillaries for the enantiomer separation of epinephrine. The CD was grafted to the double bonds of the acrylamide coating on the capillary. The length of the capillary strongly influenced the resolution of the two enantiomers of epinephrine.

Immobilised proteins have been widely used for chiral analyses, among them, α_1-acid glycoprotein (AGP) was immobilised by Hermansson in HPLC [193]. Li and Lloyd [194] showed the applicability of this chiral selector in CE for enantiomer separation of hexobarbital, pentobarbital, ifosfamide, cyclophosphamide, metoprolol, oxprenolol, alprenolol and disopyramide. The silica column (42 cm×50 μm I.D.) was filled, after preparing a frit at the end of the capillary, with a slurry composed by 5 μm particles of AGP packing material (emptying a chiral-AGP HPLC column) and a mixture of acetonitrile-phosphate buffer. The slurry was pumped at 7000 psi (4.8×10^6 Pa) and a retaining frit was made at 17 cm from the end frit. Under the operating experimental conditions (pH 3–7.5) the immobilised protein was negatively charged, as well as the capillary wall and, thus, an electro-osmotic flow was present. The mobile phase investigated contained 2 m*M* phosphate buffer and 2% organic modifier (1-propanol, 2-propanol or acetonitrile). The use of an electric field stronger than 600 caused the formation of bubbles and buffer concentrations in the range 2–10 m*M* had to be used because a current stronger than 3 μA had a negative effect on the enantiomer separation. The best chiral resolutions were obtained using a phosphate buffer at pH 6.8 and 2% of 2-propanol. The packed capillaries used were stable for at least 2 months and good reproducibility was obtained for the enantiomer separation of hexobarbital and pentobarbital (STD % of retention time, 10 runs, 2.0 and 4.3%, respectively). The electrophoretic system seems to be advantageous for sensitivity because the detector path was protein-free allowing to work at short UV wavelengths.

Recently Lloyd et al. [195] reviewed the use of proteins as chiral selectors and compared the performance of packed-capillary EC with protein phase and free-solution. EC with human serum albumin (HSA) stationary phase was compared to an AGP phase. The binding constants for HSA and analytes like promethazine, propiomazine and thioridazine were studied using a BGE supported by the protein. The enantiomer mobilities were influenced by the HSA concentration; however, the protein concentration used was relatively low (<100 μM) due to the reduction in detection signal. The measured data for binding constants had to be corrected due to the competitive effect of HSA adsorbed to the wall. In this study was also investigated the effect of a neutral additive on the HSA-drug binding constants. Since the dextran caused changes in mobilities due to the modified viscosity, the authors proposed measurements with BGE containing different concentrations of dextran with and without HSA.

Another interesting study dealing with the use of packed capillaries with β-CD CSP was performed by Li and Lloyd [196] for the chiral separation of some amino-acid derivatives, benzoin and hexobarbital. The CSP (5 μm particles, emptying a Cyclobond I HPLC column) was packed in a capillary of 50 μm I.D. The direction of the electro-osmotic flow was determined by the composition of the BGE (organic modifier, buffer and cationic additive). The presence of TEAA reversed the eof (with phosphate buffer eof was in the cathode direction). Using a phosphate buffer only benzoin and hexobarbital (neutral compounds at the operating conditions) were resolved in their enantiomers while the negatively charged analytes requested the presence of TEAA. The authors discussed about the problems arising with the use of packed capillaries, e.g., the applied electric field and the current that can cause bubble formation, and concluded that a CSP of smaller diameter packing material should be used. Table 6 shows the drugs separated in their enantiomers using EC

Table 6
Separation of chiral drugs isomers by electrochromatography

Compound	CSP	Mobile phase	Resolution	Capillary	Reference
Carprofen	β-CD (coated chirasil-dex)	20 m*M* borate–phosphate pH 7	0.94	100 (80) cm × 50 μm I.D.	[121]
Cicloprofen	β-CD (coated chirasil-dex)	20 m*M* borate–phosphate pH 7	2.32	100 (80) cm × 50 μm I.D.	[121]
Etodolac	β-CD (coated chirasil-dex)	20 m*M* borate–phosphate pH 7	1.58	100 (80) cm × 50 μm I.D.	[121]
Epinephrine	γ-CD (coated acrylamide, activated by Ce(IV))	20 m*M* borate–phosphate buffer pH 7 (10% methanol)	base-line	18 cm × 50 μm I.D.	[192]
Flurbiprofen	β-CD (coated chirasil-dex)	20 m*M* borate–phosphate pH 7	1.64	100 (80) cm × 50 μm I.D.	[121]
Hexobarbital	β-CD	4 m*M* phosphate, 5% acetonitrile pH 6.8	1.39	50 cm × 0.05 mm, 17 cm packed	[196]
		5 m*M* TEAA, 15% MeOH, pH 4.71	1.50	50 cm × 0.05 mm, 17 cm packed	[196]
	β-CD (coated chirasil-dex)	20 m*M* borate–phosphate pH 7	8.32	100 (80) cm × 50 μm I.D.	[121]
Ibuprofen	βCD (coated chirasil-dex)	20 m*M* borate–phosphate pH 7	1.97	100 (80) cm × 50 μm I.D.	[121]
	γ-CD (coated chirasil-dex)	20 m*M* borate–phosphate pH 7	0.56	100 (80) cm × 50 μm I.D.	[121]
Mephobarbital	Wall-immobilised derivatised β-CD	50 m*M* phosphate pH 7.8	base-line	65 cm × 50 μm I.D.	[197]
Oxazepam	HSA	4 m*M* phosphate pH 7 and 5% 2-propanol	–	42 (20) cm × 50 μm, 15 cm packed	[195]
Temazepam	HSA	4 m*M* phosphate pH 7 and 5% 2-propanol	–	42 (20) cm × 50 μm, 15 cm packed	[195]

4. Improving the detection limit in CE for chiral separations

Very often, due to absorption of the chiral selector added to the BGE at short wavelengths, the sensitivity of the method is relatively low and thus not useful for practical applications, and hence new strategies should be studied. An interesting approach in order to solve this problem was proposed by Valtcheva et al. [167] for the enantiomeric resolution of several β-blockers such as propranolol, pindolol, metoprolol and labetalol by CE using a BGE containing cellulase as a chiral selector; concentrations of samples as low as 10^{-5} *M* could be analysed. Fig. 11 shows that

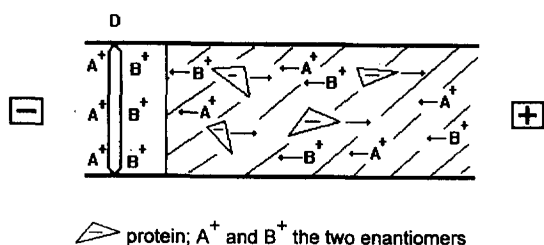


Fig. 11. Electrophoretic scheme for improving sensitivity when proteins are used as chiral selectors.

the detector cell must be chiral-selector-free and, selecting an appropriate pH, the protein is negatively charged moving in the opposite direction of the analysed basic compounds (the coated capillary will avoid disturbing caused by the electro-osmotic flow). A similar approach was also used by Terabe employing different proteins for the enantiomeric separation of compounds of pharmaceutical interest. BSA resolved homochlorcyclizine, oxyphencyclimine, propranolol, trimebutine and epinastine; ovomucoide (OVM) for bunitrolol, pindolol, arotinolol, oxyphencyclimine, tolperisone, verapamil, chlorpheniramine, primaquine and trimebutine; α_1-AGP for chlorprenaline and conalbumine for trimetoquinol [162].

A very promising approach for highly sensitive chiral analyses by CE is based on the use of on-line combination of two capillaries (for the scheme, see Fig. 12). When using a column-coupling system, the selection of the BGEs has to be done in a way that the first capillary allows to perform the chiral resolution and the second one provides an improved detection sensitivity, e.g., chiral-selector-free. The system was studied for the chiral separation of some racemic α-hydroxy acids; the first capillary contained the Cu(II)-acetate buffer and L-hydroxyproline or aspartame, while the second capillary was chiral-

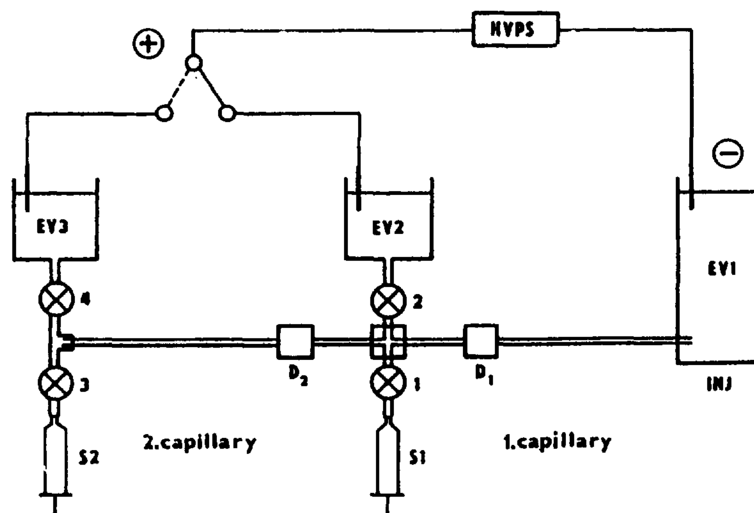


Fig. 12. Coupling column system for improving sensitivity. EV1–3, electrode vessels; D1–2, detectors; S1–2, syringes; 1–4, valves; HVPS, high voltage power supply (reprinted with permission from Ref. [198]).

selector-free. With the optimised system (aspartame as chiral selector) a detection limit of 10^{-18} *M* (sample concentration 5×10^{-7} mole) could be obtained. The detection limit recorded when both capillaries contained the chiral selector was about 10^{-15} mole (sample concentration 1×10^{-5} *M*) [198].

5. Chiral isomer analysis in biological fluids and pharmaceuticals

Only a few applications dealing with chiral drug analyses of real samples have been performed using the indirect separation method. One example was shown by Dette et al. [41] controlling the optical purity of N-acetyl-L-cysteine a drug used for the therapy of respiratory diseases. The reaction of racemic N-acetyl-cysteine with OPA and L-valine produced a couple of diastereoisomers (isoindole derivatives separated using a borate buffer at pH 8.98 containing 5% PEG. The detection limit was 0.4% of N-acetyl-D-cysteine and 99.6% of its antipode. The analyses of different pharmaceutical formulations revealed that the D-isomer was not present; the drugs were produced by stereoselective synthesis.

Enantiomer separation of racemic compounds of pharmaceutical interest was reported by us in 1989 using CZE. In this study five racemic sympathomimetic drugs, ephedrine, nor-ephedrine, epinephrine, nor-epinephrine and isoproterenol, were resolved using a phosphate buffer at pH 2.4 containing a modified CD, heptakis-2,6-di-O-methyl-β-CD. The resolution was not obtained when β-CD or tri-OMe-β-CD were employed, clearly showing that the presence of hydroxyl groups and methoxy groups at the secondary rim are necessary in order to permit chiral recognition [14].

The same chiral selector was used for the qualitative analysis of two commercial formulations, adrenaline, containing L(−)-epinephrine as a main component. The results showed the presence of less than 1%; in the second sample the presence of the D-antipode was consistent (>35%). The electropherogram of the racemic mixture of epinephrine revealed that the areas of the two peaks were different and thus it was proposed to normalize them. The differences in peak areas could be due to a different absorbance of the two analytes in the presence of the CD [119]. Later Altria et al. ascribed the differences in peak areas to the different time spent by the analytes in the detector and thus they proposed to correct them (area/migration time) [130].

A quantitative study of epinephrine in pharmaceutical formulations using di-OMe-β-CD was

shown by Peterson with good reproducibility. The peak area ratio method was used and pseudoephedrine was the internal standard. The proposed separation method was found to be reproducible and precise; the assay of pharmaceutical formulations (of different age) containing (−)-epinephrine revealed the presence of impurities in the (+)-antipode in the range 1.3–2.3% [120].

Cicletanine enantiomers in plasma were determined by CE using γ-CD as a chiral selector additive to the BGE. Cicletanine belongs to the fluoropyridine class, which is used as an antihypertensive drug. The separation and quantitation of the two enantiomers was performed after extraction with diethyl ether from human plasma. The method was linear in the concentration range 10–500 ng/ml and the detection limit was found to be 10 ng/ml for each enantiomer. Analysis of human plasma of two subjects undergoing cicletanine therapy revealed the presence of only *S*(+)-cicletanine. The analysis of urine of the same subjects after treatment of the sample with declucuronidase revealed the presence of the *R*(−)-enantiomer. The CE method was accurate and the relative error was 0.01–1.54% for *S*(+)-cicletanine and 0.87–3.2% for the *R*(−)-antipode [85].

The enantiomer separation of mephenytoin and one of its metabolite (4-hydroxy-mephenytoin was performed by Okafo et al. [91] using a mixed chiral MEKC phase. The addition of taurodeoxycholic acid (STDC) to a β-CD modified MEKC system proved superior to that of CD or STDC alone for the enantiomeric separation of the two racemic mixtures. The optimised method was applied to the analysis of a biological extract treated with hepatic microcosms. The electropherograms revealed that only the *S*-mephenytoin was transformed into *S*-4-hydroxy-mephenytoin. Thereafter, an extensive study for the analysis of enantiomers of mephenytoin, phenytoin and their metabolites (4-hydroxy-mephenytoin and 4-hydroxy-phenytoin) was done by Desiderio et al. [89] using CD-modified MEKC. The optimised method was successfully employed for monitoring the enantiotransformation of mephenytoin and phenytoin in patients under therapy.

Fig. 13 shows the MEKC analysis of mepheny-

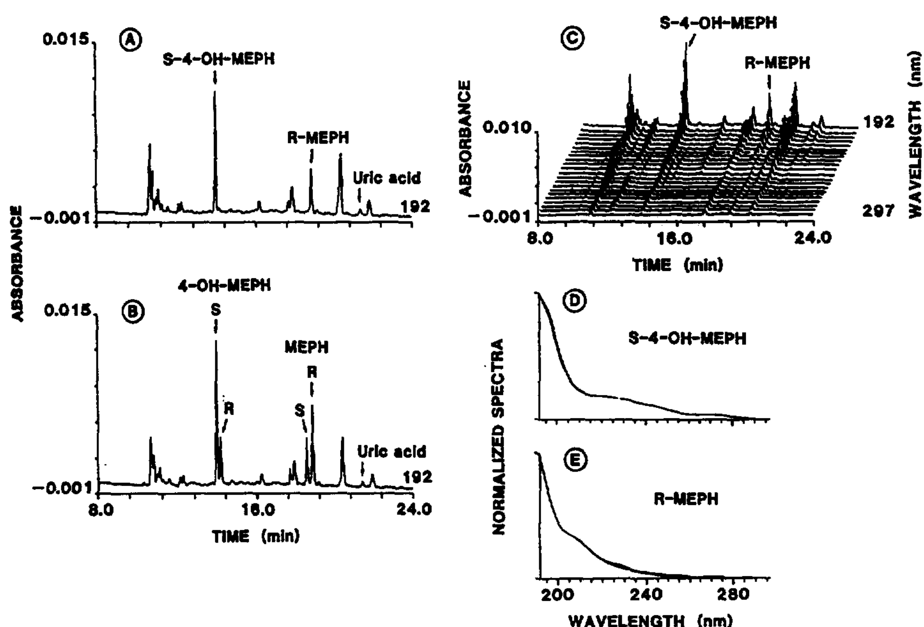


Fig. 13. CE analysis of mephenytoin and hydroxymephenytoin in urine extracted of extensive metabolizer single (A) and multi-wavelength (C). Sample spiked with racemic compounds (B) and spectral identity (D and E). Capillary, 91(54) cm × 75 μm I.D.; background electrolyte, 5.6 m*M* sodium tetraborate/8.4 m*M* Na_2HPO_4 pH 9.10, 95 m*M* SDS, 8% and 40 m*M* β-CD; applied voltage 20 kV (reprinted with permission from Ref. [89]).

toin and hydroxymephenytoin in urine extracted of extensive metabolizer patient.

Plasma samples spiked with racemic warfarin (0.4–4.0 μg/ml), after extraction with ether, was injected for the analysis of enantiomers in a BGE at pH 7 containing Glucidex 2 (a malto-oligosaccharide mixture) as a chiral selector. Acenocoumarol was the internal standard [175]. The good correlation and reproducibility obtained makes the CE a useful method for the enantiomeric analysis in biological samples. This was also demonstrated for the analysis of *R*(−) and *S*(+)-ibuprofen in serum carried out after extraction of a sample spiked with 8.5 μg/ml of racemic ibuprofen. The examined concentration was in the range reported after a single dose of 400 mg of racemic ibuprofen. The chiral selector used was maltrin M040 (96% of oligosaccharides longer than pentaose) at a concentration of 5%. Good reproducibility for either migration time (R.S.D. 1.3%) and for peak-height ratio (*S*(+)-naproxen was the internal standard, R.S.D. 1.0%). When uncoated capillary was used R(−)-ibuprofen was the first peak, while in a coated capillary the migration order was reversed [172].

In a recent study [99] CE has been applied for the chiral resolution of cationic drugs of forensic interest. The analysis was performed using a BGE containing 90% (25 m*M* Tris/H_3PO_4 pH 2.45 and 5 m*M* of di-OMe-β-CD) and 10% MeOH in a 82.5 cm fused-silica capillary (60 cm effective length)×0.05 mm I.D., 30 kV was the applied voltage. After the optimization the CE method was used for the

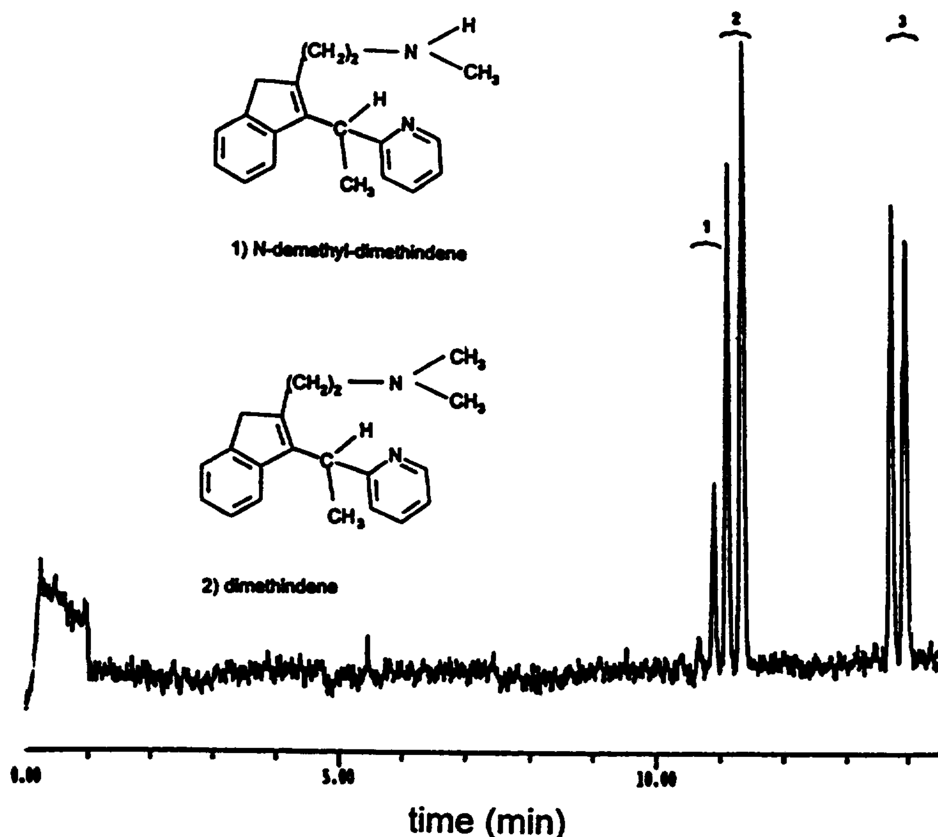


Fig. 14. Electropherogram of the enantiomer separation of dimethindene and its metabolites in urine sample (3 h after oral application of 4 mg dimethindene). 50 m*M* phosphate buffer pH 3.3 and 30 m*M* HP-β-CD; capillary 60 cm effective length × 50 μm I.D.; applied voltage 26.8 kV; detection at 205 nm (modified from Ref. [199]).

analysis of khat leave samples, revealing the presence of (−)-cathinone, (+)-norpseudoephedrine (cathine) and trace of (−)-norephedrine. In the same study the authors used 5 m*M* of di-OMe-β-CD and 1 m*M* of β-CD-SBE (IV) for the chiral resolution of amphetamine, methamphetamine and methcathinone. The analysis of forensic samples containing methcathinone and methamphetamine showed an enantiomeric ratio of 40:60 and 50:50, respectively, concluding that the (−)-cathinone was partially racemised during the illicit synthesis. The presence of unusually enantiomeric ratio can be recognised by CE analysis using CDs, e.g., in the analysis cocaine the presence of the (+)-antipode or racemic mixture indicates that the drug comes from illicit synthesis; in fact, this drug should only contain the (−)-isomer.

Trimetoquinol, a bronchodilator drug, was analysed by Nishi et al. [68] using an uncharged β-CD polymer and 0.1% of *R* antipode, the pharmacological inactive form, could be detected.

Recently Heuermann and Blaschke used CZE for the enantiomer resolution of basic racemic drugs dimethindene and its metabolite N-demethyl-dimethindene in human urine after oral administration of 4 mg of dimethindene. The optimised method was validaded and showed good sensitivity, detection limit being 1–2 ng/ml urine (signal-to-noise ratio= 3, detection at 200 nm), and good reproducibility. The appropriate chiral selector was found to be 30 m*M* HP-β-CD. To illustrate this Fig. 14 shows the electropherogram of the enantiomer separation of dimethindene and its metabolite in a urine sample 3 h after the administration of 4 mg dimethindene [199].

The analysis of nutritional supplements containing Ma Huang, made from plants in the genus Ephedra, was performed using CZE in a phosphate buffer at pH 2.4 containing HP-β-CD-(−)-ephedrine. (+)-Pseudoephedrine, (−)-N-methylephedrine and (−)-norephedrine were found and the quantitative results allowed to conclude that the ephedrines found were derived from natural sources [200].

The chiral purity control of *d*-*m*-fenfluramine was recently carried out by Porrà et al. [105] using tri-OMe-β-CD as a chiral selector added to the BGE at pH 2.5. The drug is currently used for obesity diseases and the pharmacological activity lies mainly in the D-enantiomer. The optimised CE method proved to be very reproducible (migration time R.S.D. % was 0.74 and 0.77% for the *d*- and the *l*-enantiomer, respectively, while for peak areas 1.5% was observed for both enantiomers); the detection limit for the minor component of the pharmaceutical preparation was 0.5%, measured injecting a 1.8×10^{-6} *M* solution. To illustrate this Fig. 15 shows the electropherogram of the analysis of the drug where 2% of *l*-*m*-fenfluramine was present.

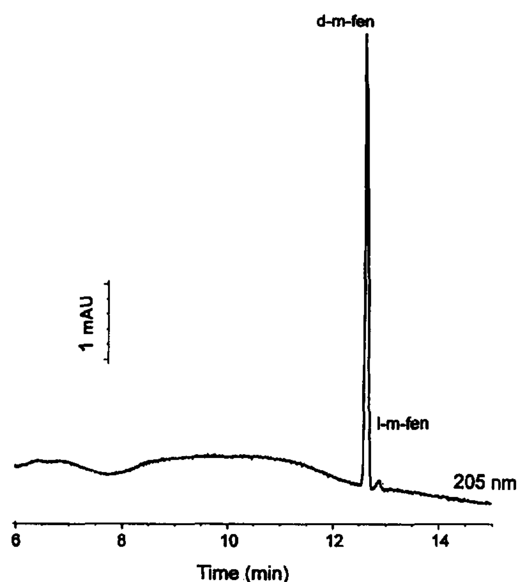


Fig. 15. Analysis of *d*- and *l*-*m*-fenfluramine in a pharmaceutical preparation where the l-antipode was present as an impurity. Hewlett-Packard HP3D; capillary 48.5(40) cm × 50 μm I.D.; 100 m*M* H_3PO_4/Tris pH 2.5 and 40 m*M* tri-OMe-β-CD; applied voltage 22 kV; capillary temperature 15°C (modified from Ref. [105]).

Table 7 summarizes the main applications in biomedical and pharmaceutical fields for the analysis of chiral drugs.

6. Conclusions

The identification of chiral drug isomers by capillary electrophoresis can be easily established, in a short period of time with good reproducibility and at a relative low cost.

Direct and indirect separation methods can be applied using a chiral environment with the aim to selectively modify the physico-chemical properties

Table 7
Biomedical and pharmaceutical applications of chiral drugs separation

Compound	Sample	CE type	Chiral Selector	BGE	Capillary	References
(−)-Cathinone, (−)-norephedrine, (−)-norpseudoephedrine	Khata edulis Forsk	CZE	di-OMe-β-CD	90% (25 m*M* Tris–H_3PO_4 pH 2.45 and 5 m*M* C.S.) and 10% methanol	82(60) cm × 50 μm I.D.	[99]
Cicletanine	plasma	CD-MEKC	γ-CD	100 m*M* sodium borate pH 8.6, 110 m*M* SDS, 10% acetonitrile and 25 m*M* C.S.	57 (50) cm × 75 μm I.D.	[85]
Clenbuterol	urine	CZE	di-OMe-β-CD	50 m*M* borax–H_3PO_4 pH 2.5 and 16 m*M* C.S.	47 cm × 50 μm I.D.	[104]
Dextrometorphan Dextrophan Levorphanol	urine	MEKC	β-CD	80% (50 m*M* sodium tetraborate, 50 m*M* HCl, 50 m*M* SDS pH 9.05 and 60 m*M* C.S) 20% 1-propanol	100(50) cm × 50 μm I.D.	[201]
Dimethindene N-dimethyl-dimethindene	urine	CZE	HP-β-CD (molar substitution (MS)=0.9)	100 m*M*; run: 50 m*M* phosphate buffer pH 3.3 and 30 m*M* C.S.	60 cm (effective length) × 50 μm I.D.	[199]
Doxylamine	cough syrup	CZE	CM-β-CD	20 m*M* citric acid pH 2.5 and 4% C.S.	27(20) cm × 75 μm I.D.	[114]
Ephedrine	urine	MEKC	(*S*)-N-dodecoxycarbonyl valine	25 m*M* Na_2HPO_4–25 m*M* $Na_2B_4O_7$, pH 8.8 and 50 m*M* C.S.	60(52.5) cm × 50 μm I.D.	[183]
	cough syrup	CZE	CM-β-CD	20 m*M* citric acid pH 2.5 and 4% C.S.	27(20) cm × 75 μm I.D.	[114]
Ephedrines [(−)-ephedrine, (+)-pseudoephedrine, (−)-N-methylephedrine, (−)norephedrine]	plant extracts	CZE/MEKC	HP-β-CD	30 m*M* Tris–H_3PO_4 and 30 m*M* HP-β-CD, 30 m*M* TMAC and 10 m*M* SDS	90 cm × 50 μm I.D.	[200]
Epinephrine	drug	CZE	di-OMe-β-CD	50 m*M* Tris–phosphate pH 2.5 and 20 m*M* C.S.	20 cm × 25 μm I.D., coated	[119]
			di-OMe-β-CD	10 m*M* Tris–H_3PO_4 pH 2.4 and 18 m*M* C.S.	50(45) cm × 75 μm I.D.	[120]

(*Continued on p. 116*)

Table 7 (*continued*)

Compound	Sample	CE type	Chiral Selector	BGE	Capillary	References
m-Fenfluramine	drug	CZE	tri-OMe-β-CD	100 m*M* H_3PO_4–Tris pH 2.5 and 40 m*M* C.S.	48.5(40) cm × 50 μm I.D.	[105]
Hexobarbital	rat plasma	MEKC	β-CD	20 m*M* phosphate buffer pH 7, 100 m*M* SDS and 30 m*M* C.S. and 15% MeOH	57 (50) cm × 75 μm I.D.	[88]
Ibuprofen	serum	CZE	Maltrin M040	30 m*M* TAPS, 10 m*M* Tris pH 7.8,5% C.S.	60 (44) cm × 50 μm I.D.	[172]
Leucovorin (5-methyl-tetrahydrofolate)	plasma	CZE	γ-CD	sodium phosphate buffer pH 9, I=0.3, 0.2 *M* C.S., 6 *M* urea	72 (50) cm × 51 μm I.D.	[202]
Mephenytoin (4-hydroxy-mephenytoin)	urine	CD-MEKC	β-CD+TDOC	30 m*M* NaH_2PO_4, 10 m*M* boric acid pH 7.2, 20 m*M* CD and 50 m*M* TDOC	57 cm × 50 μm I.D.	[91]
	urine	CD-MEKC	β-CD	5.6 m*M* sodium tetraborate and 8.4 m*M* Na_2HPO_4 pH 9.1 and 95 m*M* SDS, 40 m*M* C.S., 8% (v/v) 2-propanol	91 (54) cm × 75 μm I.D.	[89]
Trimetoquinol	drug	CZE	EP-β-CD	25 m*M* phosphate buffer pH 2.7 and 5% β-CD polymer	57 (50) cm × 75 μm I.D.	[68]
Verapamil Norverapamil	plasma	CZE	tri-OMe-β-CD	60 m*M* phosphate pH 2.5 and 60 m*M* C.S.	18 cm × 75 μm I.D.	[129]
Warfarin	serum	CZE	Glucidex 2	10 m*M* Tris–phosphate pH 7 and 2.5% C.S.	60 cm × 50 μm I.D.	[175]
	plasma	CZE	di-OMe-β-CD (degree of substitution 1.8)	100 m*M* sodium phosphate buffer pH 8.35, 8 m*M* C.S.–MeOH (98:2, v/v)	72 (50) cm × 50 μm I.D.	[139]

of the enantiomers. A large number of chiral selectors have been successfully used in CE and among them CDs and their derivatives have become the most useful. Their popularity stems from their properties: good solubility in aqueous solvents, no toxicity, very low absorbtion at relatively short, wavelengths, etc. The CE method can be applied in chiral analysis for either qualitative and quantitative purposes because good reproducibility, good detection limits are obtaineded. This can be facilitated by the development of modern instrumentation, as well as by the increasing interest in this topic. This is documented by recent work dealing with validation and optimization of CE in chiral analysis by several groups (see, e.g., Refs. [104,105,109,110,134,162, 203,204]

The method is very simple and cheap; in fact, the equilibration of the capillary is faster than with other techniques, e.g., HPLC, and the requested volume of BGE is relatively low (nl–ml range). Besides the above-mentioned advantages CE can be mainly used for analytical purposes due to the relatively low sample volume injected and thus HPLC may give better results than CE. Furthermore, specific detectors used in HPLC, e.g., polarimeteric and circular dichroism, are not yet available and thus the charcterization of the separated enantiomers in real samples is difficult.

7. List of abbreviations

AGP	α_1-acid glycoprotein
BGE	Background electrolyte
BSA	Bovine serum albumin
CD	Cyclodextrin
CDen	Mono(6-β-aminoethylamino-6-deoxy)-β-CD
CD-MEKC	CD-modified micellar electrokinetic chromatography
CE	Capillary electrophoresis
CE-β-CD	Carboxyethylated-β-CD
CGE	Capillary gel electrophoresis
CM-β-CD	Carboxymethylated-β-CD
CMC	Critical micellar concentration
C.S.	Chiral selector
CSP	Chiral stationary phase
d-cam	*d*-Camphor-10-sulfonate
di-OMe-β-CD	Heptakis(2,6-di-O-methyl)-β-CD
DOPA	Di-hydroxyphenylalanine
DTAC	Dodecyltrimethylammonium chloride
EC	Electrochromatography
FLEC	(−)-[1-(9-fluoroethyl)-ethyl]-chloroformate
GC	Gas chromatography
GITC	2,3,4,6-Tetra-O-acetyl-D-glucopyranosyl isothiocyanate
HA	Human albumin
HE	Hydroxyethylcellulose
HP-β-CD	Hydroxypropyl-β-CD
HPC	Hydroxypropyl cellulose
HPLC	High-performance liquid chromatography
HSA	Human serum albumin
HTBA	Hexadecyltrimethylammonium bromide
ITP	Isotachophoresis
l-men	*l*-methoxyacetic acid
M-β-CD	Permethylated-β-CD
MEKC	Micellar electrokinetic chromatography
MES	2-(N-morpholino)ethanesulfonic acid
NSAID	Non-steroidal anti-inflammatory drug
OVM	Ovomucoide
PEG	Poly(ethylene glycol)
PVA	Polyvinyl alcohol
SBE-β-CD	4-sulfobutyl-β-CD
SDS	Sodium dodecyl sulfate
STDC	Sodium taurodeoxycholate
TAA	Tetraalkylammonium cation
TAPS	3-[N-[Tris(hydroxymethyl)-methyl]amino]propane
TAPSO	3-[N-Tris(hydroxymethyl)-methylamino]-2-hydroxy-propanesulfonic acid
TBA	Tetrabutylammonium
TBAB	Tetrabutylammonium bromide
TLC	Thin-layer chromatography
TMA	Tetramethylammonium
TPA	Tetrapropylammonium
tri-OMe-β-CD	Heptakis(2,3,6-tri-O-methyl)-β-CD
Tris	Tris(hydroxymethyl)-amino-methane

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