Review

Controlling enantioselectivity in chiral capillary electrophoresis with inclusion–complexation

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Abstract

The separation of chiral compounds is of key importance in different fields of application, e.g., pharmaceutical, industrial, forensic, biological, clinical etc. Capillary electrophoresis (CE) is a powerful analytical method applied in chiral analysis and inclusion–complexation is one of the most frequently used mechanism to improve the selectivity of the enantiomeric separation. Cyclodextrins and their derivatives or modified crown-ethers have been successfully applied in CE for the enantiomeric separation of a wide number of analytes. This review surveys the separation of enantiomers by CE when chiral selectors, forming inclusion–complexation, are used. The control of enantioselectivity can be done carefully by considering several experimental parameters such as chiral selector type and concentration, pH, ionic strength and concentration of the background electrolyte, electroosmotic flow, organic modifier etc. The review presents a list of the latest separation of enantiomers by CE where inclusion–complexation plays a key role in the stereoselective separation mechanism. © 1997 Elsevier Science B.V.

Keywords: Reviews; Enantiomer separation; Enantioselectivity; Chiral selectors; Inclusion–complexation; Cyclodextrins

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1. Introduction

The separation of optical isomers is an important subject of research especially in the pharmaceutical field. Very often the two enantiomers of a drug exhibit different pharmacological properties, e.g., (−)-epinephrine, a sympathomimetic drug used for cardiac stimulation, is ten times more potent than its isomer [(+)-epinephrine] [1]. In some cases, one of the two enantiomers can be either inactive or even dangerous for health. Therefore analytical methods with high resolution power are requested for, e.g., chiral purity control, pharmacokinetic studies etc.

Among the analytical techniques currently used, CE is a powerful tool for the separation of both charged and/or uncharged compounds of a different nature, e.g., amino acids, peptides, proteins, herbicides, inorganic and organic ions, explosives, drugs, enantiomers etc. [2–4].

Enantiomeric separations by CE have been widely studied and the results have been reviewed by several authors [5–23].

The aim of this paper is to give an overview of chiral separations by CE using inclusion–complexation, focusing attention on the control of stereoselectivity. The effect of some parameters, such as type and concentration of inclusion–complexing agent, composition of the background electrolyte (BGE), capillary temperature etc., which influence the stereoselectivity of the chiral separations, will be discussed. Furthermore, a list of the chiral separations achieved by CE where the inclusion–complexation mechanism is involved in the separation process will be given. In order to avoid repetition, the list includes only those results obtained in late 1995–1996 and for other data, we refer to our previously published review papers [14,23].

2. Inclusion–complexing agents

2.1. Cyclodextrins and their derivatives

Cyclodextrins (CDs) are oligosaccharides constituted by several D(+)-glucopyranose units. Despite the fact that cyclodextrins with 6–12 units have been separated, only those with 6, 7 and 8 glucopyranose constituents are in frequent use in, e.g., drug composition, analytical chemistry, additives in food or tobaccos etc.

The simplest nomenclature usually used for CDs comes by the Greek alphabet and thus α, β and γ designate the oligosaccharides containing six, seven and eight glucopyranose units, respectively.

The CD shape is similar to a truncated cone with a cavity of variable dimensions (depth and width) depending on the number of glucopyranose units. The three native CDs, usually employed in analytical chemistry, possess the same depth but different widths, γ->β->α-CD.

As an example Fig. 1a Fig. 1b show the truncated cone shape and the chemical structure of β-CD, respectively; while Table 1 illustrates the main physical properties of the three native CDs.

As can be seen in Table 1, β-CD exhibits the lowest solubility in water, the most used solvent in CE, which may give some limitations to the optimization of the separation methods.

Its solubility can be increased using, e.g., either high pH or additives to the BGE, like methanol, ethanol or urea [24]. Increasing the amount of ethanol in the BGE (up to 30%), the solubility of β-CD increases, but above this value the solubility decreases. Utilizing 4 or 8 M urea solutions, it was possible to dissolve 89 or 226 mmole/liter of β-CD, respectively [25], while in the absence of urea the
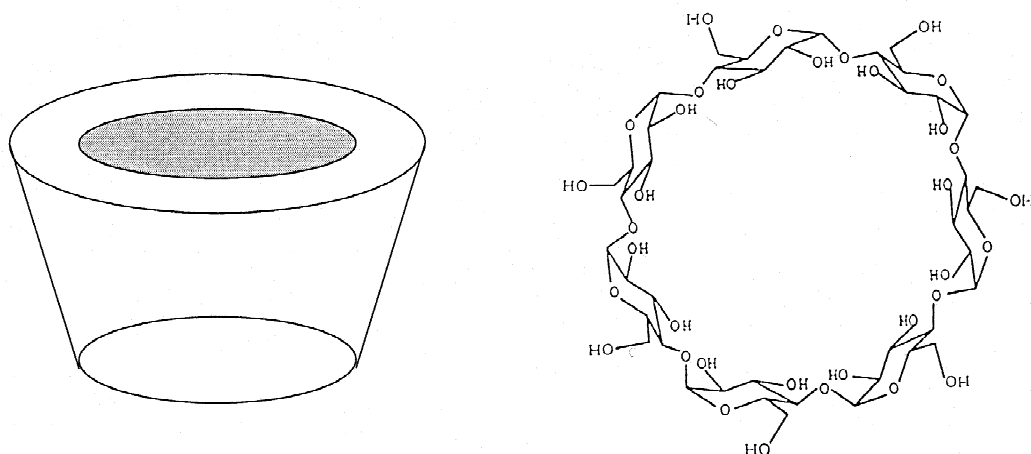
Fig. 1. (a) Chemical structure of β-cyclodextrin; (b) shape of cyclodextrin.

highest concentration of β-CD could only be of 16–20 mM.

Another way to increase the solubility of β-CD is to derivatize it and this is achieved by chemical reactions with the hydroxyl groups on the rim of the CD at positions 2, 3 and 6 of each glucose.

Based on the published data, it seems that β-CD, with the dimension of its cavity, can be applied to the analysis of a wide number of chemical compounds especially of pharmaceutical interest.

Native cyclodextrins are synthesized using starch, a natural compound, after an enzymatic reaction with glycosyltransferases or cyclodextrinases, which causes the hydrolysis of the original material [8,24] and in such a way obtains a mixture of different CDs. The pure oligosaccharides are obtained using separation methods, e.g., chromatography, crystallization, etc.

The analysis of CDs is a very important step in the synthesis process in order to verify if the compounds are pure enough for utilization in the different

application fields. Among the analytical methods so far employed for the purity control of CDs, CE with indirect UV detection seems to be very useful. As an example Fig. 2a shows the electrophoretic separation of α-, β- and γ-CD where a BGE containing an absorbing anion (benzoate) allowed the indirect detection of the analytes and improved the selectivity of the separation, forming inclusion–complexes [26] (for the separation mechanism, see Fig. 2b). A similar approach has been used by other groups for the analysis of charged CDs by CE [27–29].

As mentioned above the modification of the hydroxyl groups produces CD derivatives with a different degree of substitution influenced by several parameters such as reaction type and conditions, the ratio of the reagents etc.

The most used CD derivatives in CE, are: methylated-, hydroxyethylated-, hydroxypropylated-, acetylated-, methylamino-, carboxymethylated-, sulfated-, phosphated-, sulfobutylated-CD.

CD derivatives exhibit different properties com-

Table 1
The main properties of native cyclodextrins [24]

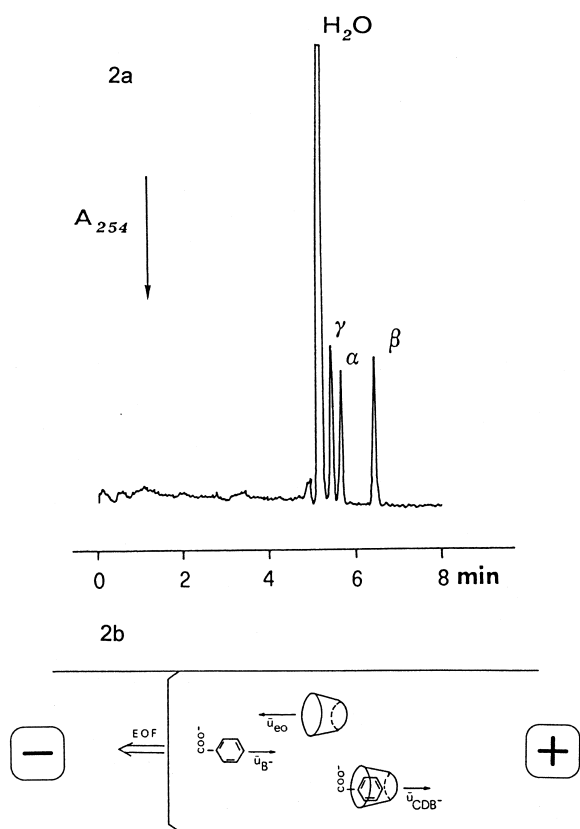| Cyclodextrin type	Number of glucopyranose	$[\alpha]_D^{25}$	Cavity (nm)		Molecular weight	Solubility (g/100 ml in water, 25°C)
			Diameter	Depth		
α	6	+150.5	0.47–0.6	0.78	972	14.5
β	7	+162.0	0.8	0.78	1135	1.85
γ	8	+177.4	1.0	0.78	1297	23.2

Fig. 2. (a) Electrophoretic separation of native cyclodextrins by indirect UV detection; (b) separation mechanism when benzoate is used as UV-absorbing ion and complexing agent. (Modified from Ref. [26].)

pared to the native one and among them we can outline the following:

1. Improved solubility,
2. Possibility for different bonds with analytes that can improve the inclusion–complexation,
3. Opportunity for the analysis of uncharged optical isomers,
4. Potentiality for reversing the migration order of two enantiomers.

For example the solubility of 2,6-di-O-methyl-β-CD and that of the negatively charged β-cyclodextrin polymer in water were found to be 57 g/100 ml [30] and >40 g/100 ml, respectively. The reported solubility values are relatively high in comparison to that of β-CD (1.85 g/100 ml). Furthermore the cavity of di-O-Me-β-CD is deeper than its parent

due to the presence of methoxy groups at the entrances and thus is more hydrophobic.

Other derivatives such as carboxymethylated, methylamino, phosphate, sulphate etc., possessing charged/chargeable groups can offer several advantages in analytical chemistry, especially in CE. In fact these type of CDs can allow the optimization of the separations, e.g., moving in the opposite direction to the analyte or inducing the charge to neutral compounds through complexation.

Finally an interesting CD derivative is represented by cyclodextrin polymers produced by reaction between CDs and polyfunctional compounds such as epichlorhydrin. In the case of this derivative, the selection of the appropriate concentrations of chemical reagents as well as the reaction conditions influences the polymer type and thus its solubility.

In CE, only soluble CD polymers have been successfully employed, namely uncharged β-CD and carboxymethylated β-CD polymers [31–35] (for a comprehensive review of CD's derivatives see Ref. [36]).

2.2. Crown-ether derivatives

Another class of compounds capable of forming inclusion–complexes is represented by crown-ether derivatives that belong to the family of macrocyclic polyethers. These compounds interact with alkaline or earth metal cations, ammonium or organic compounds with amino groups.

After the discovery of crown-ethers in 1967 by Pederson [37], 18-crown-6-ether modified with naphthyl groups has been used in HPLC [38] for chiral separations.

In CE, studies concerning host–guest complexation between metal ions and 18-crown-6-ether have been performed using ITP [39]. 18-crown-6-ether is not a chiral compound unless chiral centres are introduced by chemical reactions, e.g., the addition of four carboxylic groups to the crown molecule.

Fig. 3 shows the chemical structure of 18-crown-6-ether tetracarboxylate.

This modified crown-ether (18-crown-6-ether-tetracaboxylic acid) has been introduced in CE by Kuhn for the enantiomeric resolution of a wide number of amino compounds [7,40–45].
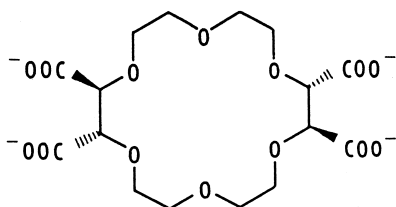
Fig. 3. Chemical structure of 18-crown-6-ether tetracarboxylic acid.

Fig. 4. Scheme of inclusion–complexation using cyclodextrins.

3. Analyte–chiral selector interaction

As mentioned above the shape of a CD is similar to a truncated cone with a relatively hydrophobic cavity, able to guest the whole molecule or part of it. The outside of the CD is relatively hydrophilic due to the presence of hydroxyl groups, primary (position 6) and secondary (position 2 and 3)

In spite of the fact that adsorption interactions, analyte–cyclodextrin, have been shown in the presence of organic solvents (this was demonstrated in HPLC experiments using 5–80% of methanol in the mobile phase) [46], the main mechanism of the CD-function involves inclusion–complexation of the analytes. The latest is based on dynamic equilibria in which water or other compounds (the analytes) replace each other, as guests, into the cavity of the cyclodextrin. Since the CD cavity possesses hydrophobic character, analytes with higher hydrophobicity exhibit higher affinity for the cavity forming more stable complexes than compounds possessing lower hydrophobicity. Secondary bonds between the analyte and the hydroxyl or modified hydroxyl groups on the rim of the CD can stabilize the inclusion–complexes formed and considering that the primary and secondary hydroxyl groups are bound to asymmetric carbons, these interactions can be stereoselective and thus responsible for the separation of enantiomeric compounds.

Fig. 4 shows a scheme of inclusion–complexation using cyclodextrins.

Knowledge of the separation mechanism in chiral analysis by CE is of paramount importance for the selection of the appropriate chiral selector in order to find the optimum experimental conditions. Recently, several authors studied the enantiomeric separation mechanism employing CDs as complexing agents by
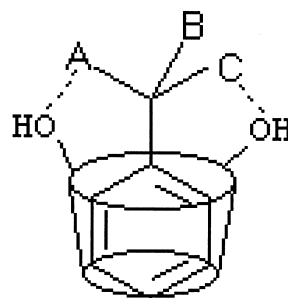
CE and in several cases the results were supported by those achieved with other analytical techniques such as NMR. The following examples reported below show that CE and NMR can be used as complementary techniques to elucidate the enantiomeric separation mechanism.

Chankvetadze et al. [47] studied the enantioseparation of mianserine (MN) (for the chemical structure see Fig. 5) and its 11 structural analogues by CE using native β-CD and three charged CD-derivatives where the nature and the position of substituents on the MN compound strongly influenced the enantiomeric resolution. The substituent at position 7, e.g., methyl had minor influence while the chlorine atom improved the chiral resolution when SBE- or SEE-β-CD were used. The authors supposed that the A and C moieties of the MN molecule were involved in the host–guest complexation; the ratios (analytes:CD) 1:1 and 1:2 were proposed. Among the CDs used, SBE-β-CD showed a higher stereoselective binding than the other chiral selectors under investigations. This was proved by ^{13}C NMR study. It was found that the signals of R-(−)-MN was shifted more than

Fig. 5. Chemical structure of minaserine derivatives.

those of S-($+$)-MN in accordance with the migration data obtained in CE. The inclusion–complexation mechanism when SBE-β-CD was used as a chiral selector in CE was also proposed by Janini et al. [48] in their recent study where several dansyl amino acids or dipeptides or chlorophenols were analyzed. The negatively charged CD was employed at pH 3.1 in coated capillaries 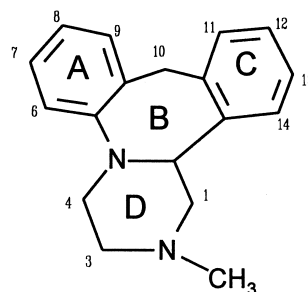with zero eof. The uncharged or weakly negatively-charged DNS-AA was incorporated into the CD cavity and the hydrogen bonds between the nitrogen groups with hydroxyl ones of the CD allowed the stereoselective resolution. This was supported by observing that the compound with the most hydrophobic residue (DNS-Trp) interacts strongly with the CD (eluted first). In the same work, the effect of the spacer was studied between the CD rim and the sulphonic group. This was done comparing SBE-β-CD and sulfated-β-CD (with and without the spacer, respectively); the last CD possesses the highest degree of substitution. Using the two CDs, it was found that DNS-Trp moves with lower migration time with SBE-β-CD than with sulfated-CD clearly showing that the highest inclusion–complexation can be achieved with the first chiral selector. The authors concluded that this effect was not due to the mobility of the CD type (sulfated-CD had higher mobility than SBE-CD) but to the fact that due to the presence of the spacer, the inclusion–complexation of analytes with SBE-β-CD was facilitated. Similar behaviour was found by Schmitt and Engelhardt [49] analyzing uncharged compounds with carboxymethylated-and carboxyethylated-β-CD. Branch et al. [50] studied the effect of acetylation of β-CD on enantioresolution of several phenethylamines by CE and NMR spectrometry. The 2,3-diacetylated CD was the best chiral selector used. NMR study suggested that the phenyl ring of phenethylamines goes into the CD cavity and the side chain interacts with the hydroxyl groups on the rim.

The separation mechanism, when crown-ethers are employed, is based on inclusion–complexation stabilized by stereoselective interactions or repulsion between the substituent groups of the asymmetric center of the analyte and carboxyl of the crown-ether derivative. As can be seen in Fig. 6, the inclusion–complexation mechanism is different than with CDs. In fact, the analyte fits the crown-ether cavity with the amino group (the hydrophilic part) on forming
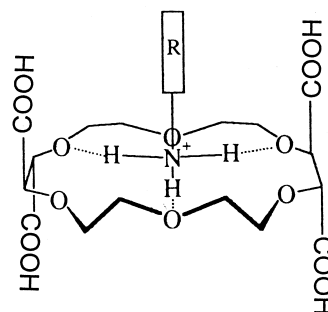
Fig. 6. Scheme of inclusion–complexation when a crown-ether derivative is used.

ion-dipole bonds with the oxygen atoms of the complexing agent. When compounds possessing non polar substituents on the asymmetric center were analyzed, e.g., methyl, phenyl etc., being incapable of forming hydrogen bonds (or electrostatic interactions), the authors proposed a different mechanism. The chiral recognition was strongly influenced by the size of the substituents on the analytes, involving steric hindrance in the separation process [43].

In a recent paper, Kuhn et al. pointed out that protonated amines form inclusion–complexes with crown-ethers analyzing di- and tripeptides and the enantioselectivity was strongly influenced by the distance between the amine and the asymmetric carbon atom [44]. Baseline resolution has been achieved for those peptides with the amino group as far as four bonds from the asymmetric center.

The affinity of an analyte towards the complexing agent (CD or crown-ether) can be recognized by the value of the association constant of the complex formed. Very often, these data are not available in the literature.

The equilibrium constants for the inclusion–complexation of mandelic acid enantiomers has been calculated by Valko et al. [51] using γ-CD and was found to be 2.8 and 2.4 M^{-1}. Recently CE has been successfully used for the calculation of association constants using electrophoretic measurements. Wren [52] calculated the equilibrium constants for propranolol and atenolol enantiomers using DM-β-CD. For atenolol, it was found that $K_{S(-)}$ and $K_{R(+)}$ were 30 and 32 M^{-1}. Using a negatively charged CD, SBE-β-CD, values of 276 and 281 M^{-1} for atenolol were calculated and the authors supposed that the different

values of the constants with the two CDs are due to the ion-pair interaction in addition to the hydrophobic one. Piperaki et al. [53] calculated the association constants of fluoxetine enantiomers with β-CD, methylated-β-CD and HP-β-CD. Gahm and Stalcup [54] determined the association constants of *N*-(3,5-dinitrobenzoyl)-amino acids and a new cyclodextrin (naphthylethylcarbamoylated-β-CD). Recently Rickard et al. [55] employed SBE-β-CD with four sulfobutyl groups for the enantiomeric separation of duloxetine at a different pH. The results achieved at pH 7 have been used for the calculation of the binding constants. The authors found good agreement between experimental and theoretical values of $\Delta\mu$; K_R and K_S were 4830 and 7820 M^{-1}, respectively. Considering the values of the association constants, we can recognize a very strong inclusion–complexation between duloxetine enantiomers and CD. The maximum enantiomeric resolution has been predicted to be 0.15 mM of SBE-β-CD whereas the measured optimum CD concentration was found to be 0.25 mM.

In their recent work, Lelievre and Gareil [56] separated the enantiomers of several arylpropionic acids (carprofen, indoprofen, ketoprofen, naproxen, pranoprofen and suprofen) using trimethylated-β-CD at pH 4. The acidity constants and the formation constants of the inclusion–complexes have been derived from electrophoretic experiments. The authors demonstrated that the knowledge of the formation constants can be useful for the interpretation of inclusion–complexation mechanism and for finding the optimum CD concentration for the studied arylpropionic acid enantiomers. Complexation constants for six derivatized amino acids (6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, AQC) have been evaluated from curve fitting procedures using β-CD and some of its derivatives [57].

Recently Fanali and Bocek [58] proposed a practical procedure for the determination of association constants of analytes–cyclodextrin equilibria by CE. The method is based on the measurement of the effective mobility of analyte at three different concentrations of chiral selector (one was zero). The mobility data were corrected for the influence of viscosity using the following formulas:

$$\mu'_A = \frac{1}{1 + K[S]}(\mu_0 + K[S]\mu'_{AS}) \tag{1}$$

$$\mu'_{AS} = \frac{[\mu'_1 C_1(\mu_0 - \mu'_1) - \mu'_2 C_2(\mu_0 - \mu'_1)]}{[\mu_0(C_1 - C_2) + \mu'_1 C_2 - \mu'_2 C_1]} \tag{2}$$

$$K = \frac{\mu_0 - \mu'_1}{\mu'_1 - \mu'_{AS}}\frac{1}{C_1} = \frac{\mu_0 - \mu'_2}{\mu'_2 - \mu'_{AS}}\frac{1}{C_2} \tag{3}$$

where μ_0 is the actual mobility of analyte A at zero concentration of CD, μ_{AS} the actual mobility of the complex CD–analyte and [S] the concentration of the chiral selector; $S = 0$ and C_1 and C_2. μ' are the corrected mobilities.

The same authors proposed a very simple method for the viscosity measurements using the thermostated CE apparatus to purge the capillary with the BGE, having the concentrations of chiral selector at 0, C_1 and C_2, at a constant pressure for a certain time, and measuring the volumes of buffer (simply weighing). The calculated association constants for L- and D-tryptophan using α-CD as the chiral selector were found to be 15.4 and 20.2 M^{-1}, respectively, with R.S.D. lower than 10%.

4. Enantiomeric resolution in capillary electrophoresis using inclusion–complexation

The separation of two compounds in CE can take place only when they move with different velocities (electrophoretic mobility) under the influence of the applied electric field. A very simple mathematical relation can be written as follows:

$$R = \frac{1}{4}\sqrt{N}\frac{\Delta\mu}{\mu_m} \tag{4}$$

where N is the number of theoretical plates (efficiency) and $\Delta\mu/\mu_m$ the selectivity, $\Delta\mu$ the difference of mobilities and μ_m the average of mobilities [59].

Considering Eq. (4), it is obvious that the increase of resolution can be achieved by increasing either efficiency and/or selectivity, however the most effective way is to modify the selectivity.

Several approaches have been used in CE in order to increase the selectivity of the separation, e.g., using an acid–base or ion-pairing equilibria, adding several modifiers to the BGE, like organic solvents, micelles, liquid gels, inclusion–complexing agents, etc. [3,19,22,23].

Cyclodextrins or modified crown-ethers have been shown to be useful compounds in CE in order to selectively modify the mobilities of analytes.

Fig. 7 shows the electrophoretic process of the separation of two charged analytes possessing the same effective mobility; the model is simplified considering the absence of eof.

Two analytes, A and B, move with their own velocities depending on their electrophoretic mobilities while the CD (uncharged) molecules behaves as a quasi stationary phase. During the electrophoretic process, the analytes interact with the CDs forming labile complexes that influence their effective mobilities. $\Delta\mu$ changes only if the two analytes have different complex formation constants with the CD molecules. The more complexed analyte is more retarded, obtaining in such a way, the resolution of A and B.

One way used to improve the selectivity of separation is represented by the use of chemical reactions with the aim of modifying the chemical structure of analytes introducing, e.g., substituent groups able to form inclusion–complexation with CDs. This is the case with free amino acids when derivatized with dansyl groups [60] or with 6-amino-quinolyl-N-hydroxysuccinimidyl carbamate (AQC) [61]. It is noteworthy to mention that the derivatization process can be advantageously used in order to improve the sensitivity. It has been shown

that the sensitivity of formoterol, a potent oral β_2-adrenoreceptor, can be increased after derivatization with fluorescein isothiocyanate and using a fluorescence detector [62]. In a recent publication, Mechref and El Rassi [63] used 7-aminonaphthalene-1,3-disulfonic acid (ANDSA) as a derivatizing agent for several phenoxy acid herbicides in order to improve the sensitivity; a laser-induced fluorescence detector was used. The ADNSA–herbicide enantiomers exhibited higher chiral resolution than the underivatized analytes in the presence of CDs in the BGE. The best results have been achieved with TM-β-CD.

4.1. Parameters affecting the stereoselectivity using inclusion–complexation

The most simple approach currently used in CE for improving the stereoselectivity can be done by modifying the background electrolyte acting on several parameters such as chiral selector type and concentration, addition of polymers or organic solvents, changing the pH and controlling the eof. The above mentioned parameters will be discussed, giving some representative examples.

4.1.1. CD type and concentration
As mentioned above, an important requirement for inclusion–complexation is that the analyte fits into
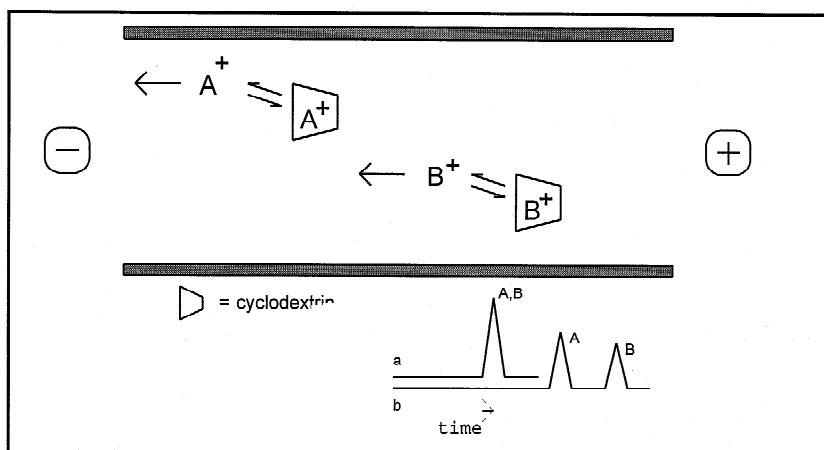

Fig. 7. Scheme of the electrophoretic separation of two cationic compounds (A and B) using cyclodextrins as chiral additives in the background electrolyte on forming labile inclusion–complexes in the absence of eof. a and b are the electropherograms in the absence and in the presence of CD, respectively.

the CD or the crown-ether cavity. Thus, the dimensions of both enantiomer and chiral complexing agent have to be carefully considered when the CE analytical method is to be optimized. The hydrophobic interaction between analyte and CD or crown cavity alone is not sufficient for the chiral recognition. Thus additional stereoselective bonds with hydroxyl or other groups on the chiral selector rim stabilize the inclusion–complexes forming diastereoisomers with different stability constants. In the simplest case (uncharged CDs), the complex with the highest affinity for the chiral selector moves with the lowest mobility.

Both mobility and enantiomeric resolution are also strongly influenced by the concentration of the chiral selector. The resolution usually increases, raising the concentration of the stereoselective agent, however an optimum concentration, where a maximum R is reached, can be found [64].

The importance of the CD type and concentration in chiral separation by CE has been shown using CGE for the separation of dansyl amino acids by Karger's group [65] or ITP for the enantiomeric separation of several compounds of pharmaceutical interest by Snopek et al. [66].

We studied the enantiomeric separation of several sympathomimetic drugs, namely ephedrine, norephedrine, epinephrine, norepinephrine and isoproterenol [67]. In this study, β-CD, DM-β-CD and TM-β-CD have been evaluated and only DM-β-CD allowed the baseline resolution of the studied compounds. The resolution increased by increasing the concentration of the chiral selector. Here the presence of both hydroxyl and methoxy groups on the rim of the CD were important for the stereoselectivity.

The effect of CD type and concentration was clearly shown for the enantiomeric separation of terbutaline comparing the results obtained using β-CD and DM-β-CD. As can be seen in Fig. 8, both CDs allowed the baseline resolution. The CD derivative showed higher stereoselectivity (maximum resolution at 5 mM) than the parent one (maximum resolution at 15 mM) [68]. The existence of the optimum concentration of CD was, later on, theoretically discussed by Wren [64]. In this theoretical model, the difference of apparent mobility of the two enantiomers can be calculated by the following expression:
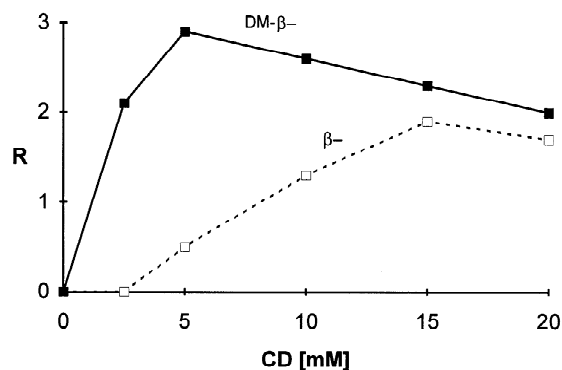
Fig. 8. Effect of cyclodextrin concentration on resolution of enantiomeric resolution of racemic terbutaline. Capillary, (polyacrylamide coated) 20 cm×0.025 mm I.D.; background electrolyte, 100 mM phosphate buffer pH 2.5 and the appropriate amount of CDs; applied voltage, 9 kV, 19 μA (constant); injection, electrokinetic, 8 kV, 10 s of 10^{-5} M of racemic terbutaline (modified from Ref. [68]).

$$\Delta\mu = \frac{(\mu_f - \mu_{com})(K_2 - K_1)C}{1 + (K_1 + K_2)C + K_1 K_2 C^2} \tag{5}$$

where μ_f and μ_{com} are the electrophoretic mobility of free and complexed analytes, respectively, K the association constants and C the concentration of CD.

Observing Eq. (5), it can be seen that $\Delta\mu$ and thus the resolution is zero or approaches to zero when C is zero or relatively high, respectively. Maximizing the difference of the free-complexed analytes mobility and the difference of the association constants, improvement of resolution can be achieved. Thus, a maximum of resolution can be expected at CD concentration $= (K_1 K_2)^{-1/2}$.

It has been discussed that the greater the association constants, the lower is the optimum selector concentration and this is shown in Fig. 9, selecting different values of K_1 and K_2 and $\mu_f = 2$ and $\mu_{com} = 1$.

The enantiomeric separation of 5,6- and 6,7-dihydroxy-2-aminotetralin was studied using several CDs such as α-, β-, γ-CD, DM-, TM-, HE-, and HP-β-CD and the most effective was the hydroxyalkylated CD modifying the BGE with uncharged and cationic additives (HPMC and HPAB). The concentration of CD was a very important parameter for the resolution of 6,7-dimethoxy-2-aminotetralin (ADMTN) enantiomers, in fact the optimum CD

$\Delta\mu$
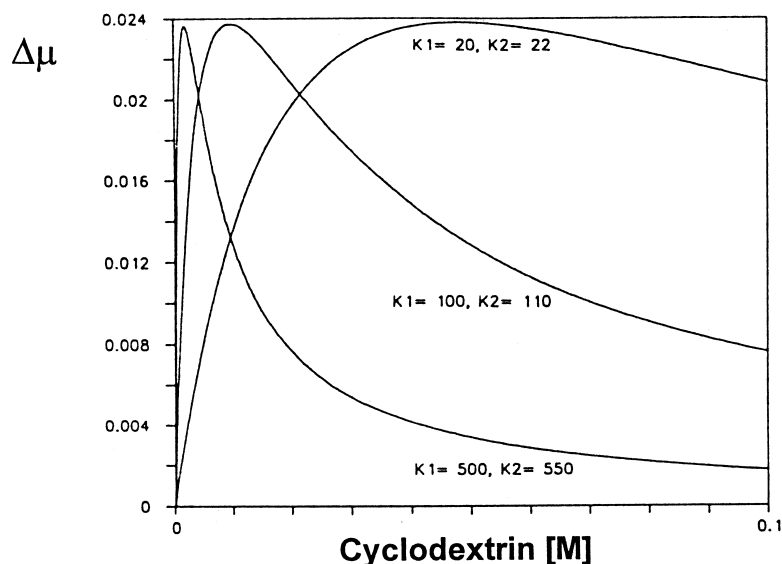
Fig. 9. Effect of CD concentration on differences of electrophoretic mobilities of two enantiomers (modified from Ref. [64]).

concentration was found at 180 mM of hydroxyalkyl-β-CD. This compound was weakly complexed (small increase of migration time and thus small affinity) [69].

The influence of the CD type and concentration on the enantiomeric resolution of 1,1-bi-2-naphthol, 1,1′-binaphthyl dihyl hydrogen phosphate and 1,1′-binaphthyldicarboxylic acid has also been discussed by Copper et al. [70]. The observed separation obtained by CE was supported by the model of enantiomeric separation. Molecular modeling has been employed in order to understand the importance of hydrogen bonding in the inclusion–complexation between several CDs and studied compounds. CD type (α-, HP-α-, DM-β-, β- and HP-β-CD) and concentration influenced the enantiomeric resolution of amphetamine and its derivatives and HP-β-CD allowed the best resolution for D,L-amphetamine [71].

Weseloh et al. [72] studied the influence of seven different methylated β-CD on the enantiomeric resolution of six basic compounds of pharmaceutical interest, the purity of the CDs used were verified by GC–MS and MALDI-TOF mass-spectroscopy. In this study, the importance of the degree of substitution of the CDs on chiral resolution has been clearly shown. In a similar study, Valko et al. [73] used HP-β-CD with a degree of substitution of 3–7.3

(DS), for the separation of derivatives of hydroxy acid enantiomers. The CD with the highest DS showed the lowest enantioresolution at the highest concentration.

We studied the effect of CD type and concentration on the resolution of erythro-and threo-mefloquine enantiomers verifying also some validation parameters. Among the studied CDs (β-CD, DM-β-CD, TM-β-CD and CM-β-CD), the dimethylated chiral selector exhibited very good stereoselectivity for the two studied racemic mixtures [74].

Tables 2 and 3 report the enantiomeric separations achieved by CE using uncharged CDs and crown-ether derivatives, respectively.

4.1.2. Effect of the charge of the chiral selector

An interesting way for controlling the enantioselectivity in chiral CE with inclusion–complexing agents is represented by the use of charged cyclo-dextrins. Modifying native CDs with charged/chargeable groups such as methylamino, carboxylic, sulfobutyl, sulfopropyl, sulphate etc., a chiral selector, with different properties than the parent CD, can be obtained and may be advantageously used in CE. The modified CD moves, under the influence of an electric field, with its own mobility, playing an important role in the chiral separation mechanism.

Table 2
Enantiomeric separations achieved using uncharged cyclodextrins or their derivatives

Compounds	CE type	CD	BGE	References
1,1′-Binaphthyl-2,2′-dicarboxylic acid (BNC)	CZE	CD- (m*M*) α- (20), Glu-α- (20), HP-β- (3%), γ- (20), HP-γ- (3%)	25 m*M* phosphate buffer pH 10.5	[87]
1,1′-Binaphthyl-2,2′-dihyl hydrogenphosphate (BNP)		β- (20+urea), TM-β- (20), HP-β- (3%), Glu-β- (20), Mal-β- (20), γ- (20), HP-γ- (3%)		
1,1′-Bi-2-naphthol	CD/MEKC	CD- (m*M*) α- (10)	25 m*M* phosphate buffer pH 8 and 50 m*M* SC	[87]
1,1′-Binaphthyl-2,2′-dicarboxylic acid (BNC)				
1,1′-Binaphthyl-2,2′-dihyl hydrogenphosphate (BNP)				
1,1′-Bi-2-naphthol	CD/MEKC	CD- (m*M*) α- (20), γ- (20), HP-β- (20)	25 m*M* phosphate buffer pH 8 and 50 m*M* SDC	[87]
1,1′-Binaphthyl-2,2′-dicarboxylic acid (BNC)		α- (20), HP-β- (20)		
1,1′-Binaphthyl-2,2′-dihyl hydrogenphosphate (BNP)		α- (20), γ- (20), HP-β- (20)		
1,1′-Bi-2-naphthol	CD/MEKC	CD- (m*M*) γ- (20)	25 m*M* phosphate buffer pH 8 and 50 m*M* SDS	[87]
1,1′-Binaphthyl-2,2′-dihyl hydrogenphosphate (BNP)		γ- (20)		
1,1′-Bi-2-naphthol	CZE	CD- (m*M*) HP-α- (3%), TM-β- (20)	25 m*M* phosphate buffer pH 10.5	[87]
ψ-Ephedrine	CZE	HP-β-CD	30 m*M* Tris-phosphoric acid pH 2.2 and 30 m*M* CD	[144]
1,1′-Bi-2-naphthol	CZE	γ-CD	10 m*M* sodium phosphate–6 m*M* sodium borate pH and 10 m*M* CD	[70]
1,1′-Binaphthyl-2,2′-diamine	CZE	β-CD	50 m*M* phosphate buffer pH 3	[28]
1,1′-Binaphthyl dihyl hydrogen phosphate	CZE	β-CD	10 m*M* sodium phosphate–6 m*M* sodium borate pH and 10 m*M* CD	[70]

Table 2. Continued

Compounds	CE type	CD	BGE	References
1,1′-Binaphthyldicarboxylic acid	CZE	α-CD	10 mM sodium phosphate–6 mM sodium borate pH and 10 mM CD	[70]
1-(4-Methoxyphenyl)(N-phenyl)ethylamine	CZE	methylated β-CD (at position 3 or 3,6)	30 mM Tris/phosphoric acid pH 2.2 and 16.5–30 mM CD	[72]
3,4-dihydro-2-H-1-benzopyran derivatives	CZE	β-CD	50 mM phosphate-borate pH 7, 8 M urea and 1–18.2 mM CD	[145]
5,6-dihydroxy-2-aminotetralin 6,7-dihydroxy-2-aminotetralin dimethoxy analogous	CZE	HP-β-CD or HE-β-CD	150 mM phosphate buffer pH 2.5, 30 mM HPAB, 0.1% HPMC and 10–180 mM CD	[69]
Adrenaline	CZE	β-CD or methylated β-CD (at position 2,6)	30 mM Tris–phosphoric acid pH 2.2 and 16.5–30 mM CD	[72]
Amino-glutetimide	CZE	γ-CD-SBE or γ-CD	40 mM phosphate pH 3, and 1 mM CD-SBE or 20 mM CD	[99]
Amino acids [N-(3,5-Dinitrobenzoyl)-phenylglycini, phenylalanine, homophenylalanine]	CZE	β-CD or NEC-β-CD	50 mM Na$_2$HPO$_4$ pH 6.5	[54]
Amino acids [1-(9-fluorenyl)-ethyl chloroformate, FLEC-Ala, Val, Ser, Met, Leu, Phe, Trp]	CD-MEKC	γ-CD	5 mM sodium borate, 25–150 mM SDS pH 9.2 and 40 mM CD	[146]
Amino acids (Dansyl amino acids, DNS-Leu)	CZE	γ-CD	25 mM phosphate buffer pH 8 and 10 mM CD	[147]
Amino acids [2-(9-Anthryl)ethyl chloroformate, AEOC-Ala, Glu, Ile, Leu, Met, Nleu, Nval, Phe, Ser, Thr, Trp, Val]	CD-MEKC	β-CD (45 mM) or γ-CD (10–25 mM, no urea)	50 mM phosphate pH 7.5, 40 mM SDS, 1 M urea, 15% 2-propanol and CD	[148]
Amino acids (DNS-Aba, Asp, Glu, Leu, Met, Nleu, Nval, Phe, Ser, Thr, Trp, Val)	CZE	β-CD	100 mM CD in N-methylformamide (NMF) and formamide (FA), 10 mM NaCl	[125]
Amino acids (N-tert.-Butoxycarbonyl, N-t-Boc) (alanine, asparagine, glutamine, histidine, isoleucine, leucine, methionine, 3-(2-naphthyl)-alanine, phenylalanine, proline, tryptophan, tyrosine, valine)	CZE	HP-β-CD	50 mM phosphate buffer pH 7.0, 15% methanol and 10 mM CD	[149]

Table 2. Continued

Compounds	CE type	CD	BGE	References
Amino acids Dansyl(leucine, norleucine, valine, norvaline, *tert*. leucine, phenylalanine, threonine, glutamate, methionine)	CZE or CD-MEKC	SBE-γ-CD or γ-CD-SDS	30 mM phosphate pH 7, methanol 10% and 5 mM SBE-CD or 20 mM CD–50 mM SDS	[99]
Amino acids (Tryptophan)	CZE	α-CD	25 mM triethanolamine-phosphoric acid pH 2.5–3.1 and 25–100 mM CD	[150]
(Tryptophan)	CZE	α-CD	100 mM phosphoric acid–Tris, Tris(hydroxymethyl) amino methane pH 2.5 and 40 mM CD	[58]
Amino acids (DNS-Leu)	CZE	γ-CD	25 mM phosphate buffer pH 8 and 10 mM CD	[147]
Amino acids (DNS-Phe)	CZE	HP-β-CD	100 mM Tris–boric acid pH 8.35, 1 mM CTAB and 1% CD or the same BGE with 5 mM spermidine (spermine), without CTAB and 0.7% CD	[83]
Amino acids (DNS-Asp, Glu, Thr, Val, Leu, Met, nor-Leu, nor-Val, Phe, Ser, Trp)	CZE	β-CD or TM-β-CD or γ-CD or DM-γ-CD or TM-α-CD	100 mM sodium borate-50 mM sodium phosphate pH 9 and 10 mM CD (effect of urea)	[151]
Amino acids (DNS-Ala, Ser, Lys, Asn, Leu, norVal, Asp, Met, Thr, Val.)	CZE	β-CD	50 mM NaH$_2$PO$_4$–100 mM borate pH 9 (compared with *N*-methylformamide and 10 mM NaCl)	[152]
Amino acids [3,5-Dinitrobenzoyl (3,5-DNB-phenylglycine, homophenylalanine)]	CZE	NEC-β-CD	50 mM Na$_2$HPO$_4$–phosphoric acid pH 6.5 and 6–7 or 8.4 mM CD	[54]
Amino acids (DNS-aminobutyric, Asp, Glu, Leu, Met, nor-Leu, nor-Val, Phe, Ser, Thr, Trp, Val)	CZE	γ-CD or methylated-γ-CD (position 2 or 3 or 6) or DM-γ-CD (position 2, 3 or 2,6 or 3,6) or TM-γ-CD (position 2,3,6)	100 mM sodium borate–50 mM sodium phosphate pH 9 and CD	[153]
Amino acids (9-fluorenylmethyl-chloroformate, FMOC-Arg, Asp, Glu, His, Ile, Leu, Met, Nleu, Nval, Phe, Pro, Ser, Thr, Trp, Val)	CD-MEKC	β-CD	45 mM phosphate pH 7, 15 mM SDS, 15% isopropanol and 12 mM CD	[119]

Table 2. Continued

Compounds	CE type	CD	BGE	References
Amino acids [6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC)-Ala, Val, Leu, Ile, Met, Pro, Cys, Lys, Ser, Thr, Asn, Gln, Phe, Trp, Tyr, His]	CZE	β-CD or β-CD polymer or HP-β-CD or DM-β-CD or TM-β-CD	10 mM borate buffer (BTP) pH 7 and 5 mM CD	[61]
Aminophosphonic acids	CZE	β-CD	100 mM borate buffer pH 9.35 and 5 mM CD	[154]
Amphetamine	CZE	DM-β-CD	50 mM Tris–H$_3$PO$_4$ pH 2.3 and 12 mM CD	[155]
Amphetamine	CZE	β-CD or methylated β-CD (at position 2)	30 mM Tris/phosphoric acid pH 2.2 and 16.5–30 mM CD	[72]
Amphetamines (Amphethamine, methamphetamine, 3,4-methylenedioxy-amphetamine, 3,4-methylenedioxymeth-amphetamine, 3,4-methylenedioxyethyl-amphetamine)	CZE	HB-β-CD	200 mM phosphate buffer pH 2.5 and 20 mM CD	[71]
Anisodamine	CZE	HP-β-CD	100 mM Tris–phosphoric acid pH 2.3 and 30 mM CD	[156]
Arterenol	CZE	DM-β-CD	20 mM phosphate buffer pH 2.2 and 30 mM CD	[83]
Aryl propionic acids (APA) (Carprofen, flurbiprofen, indoprofen, ketoprofen, pranoprofen, suprofen)	CZE	TM-β-CD	117.2 mM formic acid–1 M NaOH pH 4 and 10 mM CD	[56]
Binaphthyl derivatives (1,1′-Binaphthyl-2-2′-diamine)	CZE	β-CD or HP-β-CD or TMA-β-CD or CM-β-CD or CM-β-CD	50 mM KH$_2$PO$_4$ pH 3.3 and 1–2.5 mg/ml CD	[157]
1,1′-Binaphthyl-2,2′-diyl-hydrogen phosphate	CZE	β-CD or HP-β-CD or TMA-β-CD or CM-β-CD or CM-β-CD	50 mM KH$_2$PO$_4$ pH 3.3 and 1–2.5 mg/ml CD and pH 6	[89]
Bupivacaine	CZE	DM-β-CD	100 mM phosphoric acid–triethanolamine pH 3 and 5 mM CD	[158]
Bupivacaine	CZE	β-CD polymer	200 mM phosphate buffer pH 2.7 and 10–200 mM CD	[34]
Butaclamol	CZE	SBE-γ-CD	40 mM phosphate pH 3 and 0.1 mM SBE-CD	[99]
Carnitine FMOC (9-Fluorethylmethyl chloroformate)	CZE	DM-β-CD or γ-CD	20 mM phosphate pH 3.4–4.55 and 1–40 mM CD	[159]

Table 2. Continued

Compounds	CE type	CD	BGE	References
Carvedilol	CZE	β-CD	100 mM NaH$_2$PO$_4$–H$_3$PO$_4$ pH 2.5 and 15 mM CD	[160]
Chlophedianol	CZE	β-CD	150 mM citric acid– 100 mM Tris pH* 5.1 and 100 mM CD in formamide	[90]
Chlorphenamine	CZE	β-CD	100 mM NaH$_2$PO$_4$–H$_3$PO$_4$ pH 2.5 and 15 mM CD (no baseline separation)	[160]
	CZE	DM-β-CD or HP-β-CD	100 mM phosphoric acid–triethanolamine pH 3 and 5 mM CD	[158]
Chlortalidone	CEC	HP-β-CD (3 μm ODS or 5 μm CD bonded phase)	CH$_3$CN–5 mM phosphate pH 6.5 and 10 mM CD or without CD	[143]
Clenbuterol	CZE	β-CD or methylated β-CD (at position 2 or 3,6)	30 mM Tris–phosphoric acid pH 2.2 and 16.5–30 mM CD	[72]
Clidinium bromide	CZE	α-CD	100 mM phosphate buffer pH 2.5 and 15 mM CD	[161]
Denopamine	CZE	DM-β-CD or TM-β-CD or AC-β-CD	25 mM phosphate buffer pH 2.5, urea 2 mM and 20 mM CD	[128]
Dimethindene	CZE	DM-β-CD or HP-β-CD	100 mM phosphoric acid–triethanolamine pH 3 and 5 mM CD	[158]
Dioxypromethazine	CZE	β-CD	20-40 mM Tris–H$_3$PO$_4$ pH 2.5 and 20 mM CD	[112]
Doxylamine	CZE	DM-β-CD	20 mM phosphate buffer pH 2.2 and 30 mM CD	[83]
Duloxetine and its impurities	CZE	β-CD; DM-β-CD; TM-β-CD; HP-β-CD; HE-β-CD; SBE-β-CD	50 mM phosphate buffer pH 2.5 and 100 mM CD (5 mM HP-β-CD) (SBE-β-CD, several pHs)	[55]
Ephedrine	CZE	DM-β-CD or HP-β-CD	100 mM phosphoric acid–triethanolamine pH 3 and 5 mM CD	[158]
Ephedrine	CZE	DM-β-CD	45 mM Tris pH 2.4 and 18 mM CD	[162]

Table 2. Continued

Compounds	CE type	CD	BGE	References
Ephedrine	CZE	DM-β-CD	5 m*M* phosphate or Tris–formic acid pH 2.5–3 and 5 m*M* CD	[163]
Ephedrine	CZE	DM-β-CD	20 m*M* phosphate buffer pH 2.2 and 30 m*M* CD	[83]
Ephedrine	CZE	HP-β-CD	30 m*M* Tris–phosphoric acid pH 2.2 and 30 m*M* CD	[144]
Epinephrine	CZE	DM-β-CD	45 m*M* Tris pH 2.4 and 18 m*M* CD	[162]
Erythro-mefloquine Threo-mefloquine	CZE	DM-β-CD (or CM-β-CD for erythro)	100 m*M* phosphate buffer pH 2.5 and 2.5–10 m*M* of CD	[74]
Ethopropazine	CZE	HP-β-CD	150 m*M* citric acid– 100 m*M* Tris pH* 5.1 and 100 m*M* CD in formamide	[90]
Fenfluramine	CZE	DM-β-CD	100 m*M* phosphate pH 3, 30% methanol and 15 m*M* CD	[164]
Fenfluramine	CZE	DM-β-CD	100 m*M* phosphoric acid–triethanolamine pH 3 and 5 m*M* CD	[158]
Fenoprofen	CZE	β-CD	50 m*M* acetate buffer or 200 m*M* MES pH 4.66 and 10 m*M* CD	[147]
Formoterol (FITC derivative, fluorescein isothiocyanate)	CZE	TM-β-CD	67 m*M* phosphate buffer pH 8, 10% methanol and 20 m*M* CD	[62]
Glycopirronium	CZE	HP-β-CD or γ–CD	100 m*M* Tris–phosphoric acid pH 2.3 and 12 m*M* CD	[156]
Haloperidol (reduced, RHP)	CZE	DM-β-CD or α-CD or HP-β-CD or TM-βCD or 6-*O*-α-maltosyl- α-CD	40 m*M* phosphate buffer pH 2.5 and 5 or 10 m*M* CD	[165]

Table 2. Continued

Compounds	CE type	CD	BGE	References
Herbicides (chlorophenoxya cids)	CZE	α-CD or β-CD	phosphate buffer pH 5.6 and 1–10 mM CD (chiral and no chiral separations)	[166]
Herbicides (acid) (7-Aminonaphthalene-1,3-disulfonic acid derivatives, ANDSA)	CZE	α-, β-, γ-, HP-β-, DM-β-, TM-β-CD	200 mM borate pH 10 or phosphate-borate buffer pH 5 5–10 mM CD	[63]
(2,4-dichlorophenoxy)acetic acid; (2,4,5-trichlorophenoxy)acetic; 2-phenoxypropionic; 2-(4-chloro-2-methylphenoxy)-propionic; 2-(2-chlorophenoxy)propionic; 2-(2,4-dichlorophenoxy)propionic; silvex			(chiral and non-chiral separations)	
Hexobarbital and hydrolysis product	CZE	HP-β-CD or β-CD	100 mM Tris–boric acid pH 8.35, 1 mM CTAB and 1% CD or the same BGE with 5 mM spermidine (spermine), without CTAB and 0.7% CD	[83]
Homatropine	CZE	HP-β-CD	25 mM phosphate buffer pH 2.5 and 10 mM CD	[147]
Hydroxyacids (Mandelic, m-hydroxy-mandelic, p-hydroxy-mandelic, 3,4-di-hydroxy-mandelic, 2-phenyllactic, 3-phenyllactic)	CZE	β-CD polymer	50 mM Na$_2$HPO$_4$–NaOH pH 6 and 20–100 mg/ml of CD	[33]
Ibuprofen	CZE	β-CD	400 mM MES buffer pH 4.66 and 15 mM CD	[167]
Isoprenaline	CZE	DM-β-CD or HP-β-CD	100 mM phosphoric acid–triethanolamine pH 3 and 5 mM CD	[158]
Isoproterenol	CZE	β-CD polymer	25 mM phosphate buffer pH 2.7 and 10–50 mg/ml of CD	[34]
Isoproterenol	CZE	DM-β-CD	45 mM Tris pH 2.4 and 18 mM CD	[162]
Isoproterenol	CZE	HP-β-CD or M-β-CD (DS not declared)	100 mM litium acetate pH 4.75 0.1 g/ml CD	[106]

Table 2. Continued

Compounds	CE type	CD	BGE	References
Isoproterenol	CZE	DM-β-CD	25 mM phosphate buffer pH 2.5 and 10–50 mM CD	[168]
Ketamine	CZE	β-CD	100 mM NaH$_2$PO$_4$–H$_3$PO$_4$ pH 2.5 and 15 mM CD (no baseline separation)	[160]
Ketamine	CZE	α-CD	100 mM phosphate buffer pH 2.5 and 15 mM CD	[161]
LY231514 (N-[4-[2-(2-amino-4,7-dihydro-4-oxo-1H-pyrollo[2,3-d]pyrimidin-5-yl)ethyl]-L-glutamic acid)	CZE	β-CD	400 mM borate pH 9.1 and 2.5 mM CD	[169]
Mefenorex	CZE	TM-β-CD	30 mM Tris–phosphoric acid pH 2.2 and 16.5–30 mM CD	[72]
Metaproterenol	CZE	β-CD or HP-β-CD or DM-β-CD	25 mM phosphate buffer pH 2.5 and 3–100 mM CD	[167]
Metaproterenol	CZE	DM-β-CD	25 mM phosphate buffer pH 2.5 and 10–50 mM CD	[168]
Methadone and its primary metabolite (2-Ethylidene-1,5-dimethyl-3,3-di-phenylpyrrolidone, EDDP	CZE	HP-β-CD	100 mM KH$_2$PO$_4$ buffer pH 3 and 4.3 mM CD	[170]
Methamphetamine	CZE	β–CD-polymer	10–200 mM phosphate buffer pH 2.5–6 and 200 mg/ml of CD	[136]
Methoxyphenamine	CZE	β-CD-polymer	25 mM phosphate buffer pH 2.5 and 2–50 mM CD	[167]
Methomidate (MET)	CZE	β-CD or γ-CD or HP-β-CD or Me-β-CD or SBE-β-CD or SEE-β-CD or CM-β-CD	50 mM KH$_2$PO$_4$/50 mM Na$_2$PO$_4$ pH 3.5 or 6	[171]
Mianserine	CEC	HP-β-CD (5 μm C.S. bonded phase)	CH$_3$CN–10 mM phosphate pH 7.5 without CD (no baseline separation)	[143]
Mianserine	CZE	β-CD or HP-β-CD	150 mM citric acid–100 mM Tris pH* 5.1 and 100 mM CD in formamide	[90]

Table 2. Continued

Compounds	CE type	CD	BGE	References
Muscarone and muscarinic antagonistics	CZE	β-or γ-CD	25 m*M* Tris–acetate buffer pH 4.2 and 10–30 m*M* CD	[172]
N-(2-Phenyl-1-hydroxy-ethyl)-*N,N,N*-tributyl ammonium	CZE	β-CD	50 m*M* phosphoric acid–tetraethylammonium hydroxide pH 2.2 and 15 m*M* CD	[105]
N-Methyl-ψ-ephedrine	CZE	HP-β-CD	30 m*M* Tris–phosphoric acid pH 2.2 and 30 m*M* CD	[144]
N-Methylephedrine	CZE	HP-β-CD	30 m*M* Tris–phosphoric acid pH 2.2 and 30 m*M* CD	[144]
Naproxen	CZE	β-CD or HP-β-CD or DM-β-CD	25 m*M* sodium acetate pH 4.66 and 3–100 m*M* CD 25 m*M* phosphate buffer pH 2.5 and 3–50 m*M* CD	[167]
Nefopam	CZE	β-CD or HP-β-CD or TM-β-CD	150 m*M* citric acid–100 m*M* Tris pH* 5.1 and 100 m*M* CD in formamide	[90]
Norephedrine	CZE	DM-β-CD	45 m*M* Tris pH 2.4 and 18 m*M* CD	[162]
Norephedrine	CZE	HP-β-CD	30 m*M* Tris–phosphoric acid pH 2.2 and 30 m*M* CD	[144]
Norepinephrine	CZE	DM-β-CD	45 m*M* Tris pH 2.4 and 18 m*M* CD	[162]
Orciprenaline	CZE	β-CD	100 m*M* NaH_2PO_4–H_3PO_4 pH 2.5 and 15 m*M* CD (no baseline separation)	[160]
Orphenadrine	CZE	α-CD	100 m*M* phosphate buffer pH 2.5 and 15 m*M* CD	[161]
Oxomemazine	CZE	α-CD	100 m*M* phosphate buffer pH 2.5 and 15 m*M* CD	[161]

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Table 2. Continued

Compounds	CE type	CD	BGE	References
Oxprenolol and their basic metabolites	CZE	HP-β-CD	100 mM NaH$_2$PO$_4$–H$_3$PO$_4$ pH 2.5, 0.05 PEG, 0.03 mM TBA and 50 mM CD	[173]
Peptides (9-Fluorenylmethyl chloroformate, FMOC-Ala-Ala, Ala-Gly, Ala-Gly-Gly, Ala-Leu, Gly-Ala, Gly-Asn, Gly-Asp, Gly-Leu, Gly-Met, Gly-Phe, Gly-Val, Leu-Ala, Leu-Gly, Leu-Gly-Gly, Leu-Leu)	CD-MEKC	γ-CD or β-CD	40 mM phosphate buffer pH 7.5, 40 mM SDS, 15% 2-propanol and 12 mM CD	[120]
Phenethylamines (Norephedrine, ephedrine, methylephedrine, 4-hydroxynorephedrine, 4-hydroxyamphetamine, oxilofrine, pholedrine, oxedrine, norfenefrine, etilefrine, orciprenaline, terbutaline)	CZE	2,3-diac-β-CD	0.1 M potassium dihydrogen phosphate buffer pH 4.5 and 12 mM CD	[50]
Phenylalanine methyl ester	CZE	β-CD	25 mM sodium borate pH 2.5	[174]
Phenylalkylamine (deprenyl, propargyl-amphetamine, *p*-fluoro-deprenyl, methamphetamine, *p*-fluoro-methamphetamine, amphetamine, ephedrine, ψ-ephedrine, norephedrine	CZE	DM-β-CD	20 mM Tris-phosphate pH 2.7, 0.5% hydroxypropylmethyl cellulose (HPMC) and 0–48 mM CD (effect of organic solvent)	[175]
Phenylephrine	CZE	β-CD	50 mM Tris–H$_3$PO$_4$ pH 2.3, 6 M urea and 100 mM CD	[155]
Pindolol	CZE	DM-β-CD	20 mM phosphate buffer pH 2.2 and 30 mM CD	[83]
Polychlorinated biphenyls (PBCs)	CD-MEKC	γ-CD-SDS	5 or 10 mM CHES (pH 10), 2 M urea, 110 mM SDS and 30 or 50 mM CD	[176]
Polychlorinated biphenyls (PCBs)	CD-MEKC	β-CD and γ-CD	60 mM 2-(*N*-cycohexylamino)-ethanesulphonic acid (CHES) pH 10, 2 M urea, 110 mM SDS and 73 mM β-CD +22 mM γ-CD	[177]

Table 2. Continued

Compounds	CE type	CD	BGE	References
Pseudoephedrine	CZE	DM-β-CD	45 mM Tris pH 2.4 and 18 mM CD	[162]
Quinolone drugs (Ofloxacin, DV-7751, DU-6859)	CZE	γ-CD/Zn(II)-(L or D)-Phe	10 mM ammonium acetate pH 6.5 and 5–20 mM CD	[133]
Ropivacaine	CZE/MS	DM-β-CD	1 mM formic acid pH 2.85 and 50 mM CD (transfer to MS buffer without CD)	[178]
Secobarbital	CD-MEKC or CZE	γ-CD-SDS or γ-CD-SBE	30 mM phosphate pH 7, methanol 15% and 25 mM CD–50 mM SDS or 15 mM CD-SBE	[99]
Selegiline	CZE	β-CD-polymer	10–200 mM phosphate buffer pH 2.5–6 and 200 mg/ml of CD	[136]
Sulfonamides (β-Methylsulfonamide, MPS; 2-Butylsulfonamides, BS; 2-Methylsulfonamides, MBS)	CZE	DM-β-CD or β-CD-polymer or α-CD-polymer or HP-β-CD or TM-β-CD	100 mM Tris/various anions (benzoate, maleate, fumarate, chloride, chromate) pH 8.2–8.5 and different concentrations of CD	[179]
Terbutaline	CZE	DM-β-CD	25 mM phosphate buffer pH 2.5 and 50 mM CD	[147]
Terbutaline	CZE	DM-β-CD	5 mM phosphate or Tris–formic acid pH 2.5–3 and 5 mM CD	[163]
Terbutaline	CZE	DM-β-CD	25 mM phosphate buffer pH 2.5 and 50 mM CD	[147]
Terbutaline	CZE	DM-β-CD or HP-β-CD	100 mM phosphoric acid–triethanolamine pH 3 and 5 mM CD	[158]
Terbutaline	CZE	β-CD polymer	25 mM phosphate buffer pH 2.7 and 10–50 mg/ml of CD	[34]
Tetryzoline	CZE	β-CD	100 mM NaH_2PO_4–H_3PO_4 pH 2.5 and 15 mM CD	[160]

Table 2. Continued

Compounds	CE type	CD	BGE	References
Tetryzoline	CZE	α-CD	100 mM phosphate buffer pH 2.5 and 15 mM CD	[161]
Thioridazine	CZE	β-CD or TM-β-CD or γ-CD	150 mM citric acid–100 mM Tris pH* 5.1 and 100 mM CD in formamide	[90]
Tioconazole	CZE	β-CD	100 mM phosphoric acid–7.4 M triehanolamine to pH 3.0 and 0.8–15 mM CD (effect of organic solvents)	[180]
Trans-1,2-Dihydrodiol-chrysene metabolite	CD-MEKC	γ-CD	25 mM phosphate buffer pH 7.8, 50 mM SDS, 7.4% 2-propanol and 20 mM CD	[181]
Tranylcypromine	CZE	methylated β-CD (at position 2,3 or 2,6 or 3,6)	30 mM Tris–phosphoric acid pH 2.2 and 16.5–30 mM CD	[72]
Trihexylphenidyl	CZE	TM-β-CD	150 mM citric acid–100 mM Tris pH* 5.1 and 100 mM CD in formamide	[90]
Trimepazine	CZE	β-CD or HP-β-CD or γ-CD	150 mM citric acid–100 mM Tris pH* 5.1 and 100 mM CD in formamide	[90]
Trimipramine	CZE	β-CD or HP-β-CD or γ-CD	150 mM citric acid–100 mM Tris pH* 5.1 and 100 mM CD in formamide	[90]
	CZE	β-CD	150 mM citric acid–100 mM Tris pH* 5.1 and 250 mM CD in N-methylformamide	[90]
Tropicamide	CZE	β-CD	100 mM NaH$_2$PO$_4$–H$_3$PO$_4$ pH 2.5 and 15 mM CD	[160]
Tropicamide	CZE	α-CD	100 mM phosphate buffer pH 2.5 and 15 mM CD	[161]

Table 2. Continued

Compounds	CE type	CD	BGE	References
Tryptophan esters (methyl, ethyl, butyl)	CZE	β-CD polymer	25 mM phosphate buffer pH 2.7 and 10–50 mg/ml of CD	[34]
Zopliclone	CZE	β-CD	100 mM NaH$_2$PO$_4$–H$_3$PO$_4$ pH 2.5 and 15 15 mM CD	[160]
Zoplicone	CZE	β−CD	100 mM phosphate buffer pH 2.75 (triethanolamine) 16.3 mM CD	[182]
Zoplicone N-oxide				
N-Desmethylzoplicone				

pH* = apparent pH.

Looking at Eq. (5), we can argue the advantages of using charged CDs. In fact, a higher resolution can be achieved increasing $\Delta\mu$ (mobility difference of free analyte/complexed). Another very important advantage on using charged CDs is the possibility of analysing uncharged compounds; previously such analytes were studied with CD-MEKC employing micelles (SDS) and cyclodextrins [75–77].

Several publications deal with the use of charged chiral selectors in CE [28,29,31,47–49,78–93] and only some examples will be given below in order to show the usefulness of such additives in the BGE for improving the enantioselectivity; a list of chiral separations achieved with charged CDs can be found at the end of this review (see Tables 4 and 5).

The first application on using charged CDs can be found reading the papers of Terabe's group [94]. Here the positively charged mono(6-β-amino-ethylamino-6-deoxy)-β-CD was employed for the enantiomeric separation of some dansyl-amino acids. Later on, we studied the effect of 6[A]-methylamino- and 6[A],6[B]-dimethylamino-β-CD (positively charged) on the resolution of several hydroxyacid enantiomers [95]. Schmitt and Engelhardt used carboxy-methylated-β-CD focusing on the importance of the charge on chiral recognition by CE. In this study, the modified CD can be either uncharged or negatively charged. At pH>5, the CD had its own charge. Good enantioselectivity was obtained for the separation of drugs such as hexobarbital and oxazolidinone [49]. Interesting results have been achieved by several authors using sulfobutyl-β-CD

(SBE-β-CD) [52,79,82,86,88,96] which is negatively charged in a wide range of pH. This modified CD possesses four sulfonic groups bound to position 6 of glucopyranose through the butyl chain (see Fig. 10).

Ephedrine alkaloids have been separated into their enantiomers at pH 10, a value close to the pK_a of the analytes [86]. Very low concentrations of SBE-β-CD (1.5–5 mM) have been used by Tait et al. at acidic pH=2.5 for the enantiomeric separation of ephedrine, pseudoepehdrine, adrenaline, noradrenaline and dihydroxyphenylalanine [82].

An interesting method has been shown by Blaschke's group on using SBE-β-CD; here the chiral selector was injected, as a small zone, into the capillary prior to the injection of the sample (dimethindene). Due to the counter-current effect, a very low concentration of CD (<40 μM) was enough for the enantiomeric separation of the compounds being studied. [79].

The effect of CD type and concentration has also been studied by Rickard et al. [55] using uncharged and negatively-charged CDs. Among the different charged CDs, very interesting results have been obtained with pure SBE-β-CD (with 4 sulfobutyl groups). Peaks tailing were observed at pH 2.5 and the effect was remarkable increasing the number of sulfobutyl groups in the CD while at pH=4, a less pronounced peak tailing was recorded when using CD with 1 and 4 sulfobutyl substituents. The influence of the degree of subtitution on enantiomeric resolution was also studied by Szeman et al. [97] employing CM-β-CD (different DS in the range

Table 3
Chiral separations with 18-crown-6-ether tetracarboxylic acid

Compound	CE type	Complexing agent	Experimental conditions	References
5,6-Dihydroxy-2-aminotetralin	CZE	18C6TA	Buffer pH 2.2 and 10–50 mM of Crown	[183]
Amino acids [o-Fluorophenylalanine, m-fluorophenylalanine, p-fluorophemylalanine, phenylalanine, phenylglycine, tyrosine, tryptophan, hydroxytryptophan, 3-(3,4-dihydroxyphenyl)-alanine, leucine, isoleucine, valine, threonine, serine]	CGE	18C6TA and β-CD	0.3% agarose gel in 10 mM Tris–citric acid and 20 mM 18C6TA + 20 mM CD	[113]
Amino acids (Phenylalanine, DOPA, tryptophan)	CZE	18C6TA	Formamide with 2.5 mM TBAP and 25 or 50 mM Crown	[123]
Amino alcohol (Norephedrine, noradrenaline, 2-amino-1,2-diphenylethanol)	CZE	18C6TA	Formamide with 0–40 mM TBAP and 2.5–10 mM Crown	[123]
Amino compounds (Aminoglutethimide, blacofen, kynunerine, mexiletine, noradrenaline, norephedrine, octopamine, primaquine, Tyr, DOPA, alanine-β-naphthylamide, 1,1″-binaphthyl-2,2″-diamine)	CZE	18C6TA	20 mM Tris–H_3PO_4 pH 2.06 and 10 mM of Crown (15°C)	[45]
Aromatic amines (1-Naphthylethylamine, 1-phenylethylamine)	CZE	18C6TA	Formamide with 100 mM TBAP and 10 mM Crown	[123]
Dipeptides (Alanyl-serine, alanyl-valine, leucyl-alanine, leucyl-leucine, leucyl-phenylalanine, leucyl-tyrosine, leucyl-valine)	CZE	18C6TA	10 mM Tris–citrate pH 2.2 and 25 mM Crown	[184]
(Phe-Ala, Phe-6-aminocaproic acid, Gly-Trp, Gly-Phe, β-Ala-Phe, 6-aminocaproic acid-Phe)	CZE	18C6TA	10 mM Tris–citrate pH 2.5 and 5 or 10 mM Crown	[44]

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Table 3. Continued

Compound	CE type	Complexing agent	Experimental conditions	References
Glycyl-dipeptides (Aspartate, asparagine, leucine, methionine, norleucine, norvaline, serine, threonine, tryptophan, valine, alanine, phenylalanine)	CZE	18C6TA	10 mM Tris–citrate pH 2.2 and 25 mM Crown	[184]
Tripeptides (DL-Phe-Gly-Gly, L-Ala-L-PheGly/ D-Ala-D-PheGly, Gly-DL-Leu-DL-Ala, Gly-L-Ala-L-Phe/ Gly-D-Ala-D-Phe, GlyGly-DL-Leu)	CZE	18C6TA	10 mM Tris–citrate pH 2.5 and 10 mM Crown	[44]
Noradrenaline	CGE	18C6TA and β-CD	0.3% agarose gel in 10 mM Tris–citric acid and 20 mM 18C6TA + 20 mM CD	[113]
1-(1-Naphthyl)-ethylamine	CGE	18C6TA and β-CD	0.3% agarose gel in 10 mM Tris–citric acid and 20 mM 18C6TA + 20 mM CD	[113]

2–8), dissolved in BGE at pH 8.2, concluding that the optimum DS value depends on the molecular characteristics of analytes.

We also investigated the effect of SBE-β-CD on the enantiomeric resolution of several basic and acidic racemic compounds of pharmaceutical interest as well as several dansyl-amino acids studying the effect of several parameters such as CD concentration and pH of the BGE [88]. Recently we reported the enantiomeric separation of several herbicides (uncharged and free acids) using SBE-β-CD as chiral selector. The resolution was influenced by the CD concentration and by the pH of the BGE. Bromacil, chlorbufam, napropramide, ethofumesate, imazapyr, haloxyfop free acid, flamprop free acid showed good enantiomeric resolution using 50 mg/ml of SBE-β-CD dissolved in the buffer at pH 9 [98].

The use of SBE-CD derivatives in CE has been extended by Jung and Francotte comparing the effect of SBE-γ-CD with native cyclodextrin [99]; different enantioselectivity has been shown, e.g., the resolution of dansyl-*tert*.-leucine was not achieved using

γ-CD while base-line resolution was recorded with SBE-γ-CD. Performing the same analysis with SBE-β-CD, the reversion of the migration order of the two enantiomers of the dansyl amino acid was shown.

The usefulness of negatively-charged CDs for the enantiomeric resolution of uncharged compounds has been documented by several authors. Thalidomide enantiomers were resolved using SBE-β-CD by Blaschke's group [79], phensuximide and indapamine have been resolved by Wu and Stalcup [100] employing a sulfated-β-CD while Desiderio and Fanali separated uncharged alcohols with SBE-β-CD [88]. Recently, Fillet et al. [101] separated enantiomers of acidic drugs such as sulindac, fenoprofen, ketoprofen, warfarin and hexobarbital using a BGE at pH 3 containing uncharged and charged β-CD derivatives. The complexation with the negative CD provided the analytes with an adequate mobility while the uncharged CD allowed enantiorecognition; the best enantiomeric resolutions were achieved when SBE-β-CD was used in combination of DM-β-CD.

Another interesting effect that can be achieved

Table 4
Selected enantiomeric separations using charged cyclodextrin derivatives

Compound	CE type	CD	BGE	References
Benzyl mandelate	CZE	β-CD phosphate	50 m*M* phosphate pH 6.5 and 10 m*M* CD	[92]
Benzoin	CZE	β-CD phosphate	50 m*M* phosphate pH 7 and 2.5 m*M* CD	[92]
	CZE	β-CD-phosphate (m*M*)	50 m*M* phosphate buffer pH	[92]
Chlorprenaline		0.2	5	
Imazalil		0.2	5	
Salbutamol		0.4	5	
Trimebutine		0.4	5	
Verapamil		0.4	5	
Metanephrine		0.5	5	
Ketamine		0.7	5	
Azelastine		1	7	
	CZE	β-CD-phosphate (m*M*)	50 m*M* phosphate buffer pH 5	[92]
Aminoglutethimide		3		
Norephedrine		5		
Phenylephrine		5		
Primaquine		5		
Promethazine		5		
Thioridazine		5		
	CZE	β-CD-phosphate (m*M*)	50 m*M* phosphate buffer pH	[92]
Bunitrolol		5	5	
Nicardipine		5	5	
Acebutolol		10	5	
Bupivacaine		10	5	
Sulpiride		10	5	
1,1′-Bi-2-naphthol (BN) 1,1′-Binaphthyl-2,2′-dihyl hydrogen-phosphate (BNP)	CZE	β-CD-phosphate (m*M*)	25 m*M* phosphate buffer pH 9 and 5 or 10 m*M* CD	[87]
1,1′-Binaphthyl-2,2′-dicarboxylic acid (BNC)	CZE	β-CD-phosphate (m*M*) +20 m*M* α-CD	25 m*M* phosphate buffer pH 9 and 5 m*M* CD	[87]
1,1′-Binaphthyl-2,2′-dihyl hydrogen-phosphate (BNP)	CZE	β-CD-phosphate (m*M*) +20 m*M* TM-β-CD	25 m*M* phosphate buffer pH 9 and 5 m*M* CD	[87]
1,1′-Binaphthyl-2,2′-dicarboxylic acid (BNC)				
	CZE	γ-CD-phosphate	50 m*M* phosphate buffer pH 5	[92]
Epinastine		0.1		
Primaquine		0.2		
Clorprenaline		0.5		
Chlorpheniramine		1		
Denopamine		1		
Verapamil		1		
Mexiletine		2		
Trimetoquinol		2		

Table 4. Continued

Compound	CE type	CD	BGE	References
Tolperisone		3		
Nebracetam		4		
Bisoprolol		5		
Bupivacaine		5		
Eperisone		5		
Etilephrin		5		
Fenoterol		5		
Promethazine		5		
Thioridazine		5		
Trimetoquinol		5		
Metanephrine		5		
Bunitrolol		10		
Metoprolol		10		
Chlorpheniramine	CZE	6^{A}-Methylamino-β-CD	100 mM phosphoric acid–TMA pH 2.5 and 1–10 mM CD	[91]
Isoproterenol	CZE	6^{A}-Methylamino-β-CD	100 mM phosphoric acid–TMA pH 2.5 and 1–10 mM CD	[91]
Ketamine	CZE	6^{A}-Methylamino-β-CD	100 mM phosphoric acid–TMA pH 2.5 and 1–10 mM CD	[91]
Propranolol	CZE	6^{A}-Methylamino-β-CD	100 mM phosphoric acid–TMA pH 2.5 and 1–10 mM CD	[91]
Terbutaline	CZE	6^{A}-Methylamino-β-CD	100 mM phosphoric acid–TMA pH 2.5 and 1–10 mM CD	[91]
3-Pheyllactic acid	CZE	6^{A}-Methylamino-β-CD or hepta-methylamino-β-CD	150 mM Britton Robinson buffer pH 5 or pH 7 and 1–10 mM CD	[91]
Acenocoumarol	CZE	6^{A}-Methylamino-β-CD or hepta-methylamino-β-CD	75 mM Britton Robinson buffer pH 5 or pH 7 and 1–10 mM CD	[91]
Aryl propionic acids (fenoprofen, tiaprofenic acid, 3-isomer tiaprofenic acid)	CZE	6^{A}-Methylamino-β-CD or hepta-methylamino-β-CD	75 mM Britton Robinson buffer pH 5 or pH 7 and 1-10 mM CD	[91]
Warfarin	CZE	6^{A}-Methylamino-β-CD or hepta-methylamino-β-CD	75 mM Britton Robinson buffer pH 5 or pH 7 and 1–7.5 mM CD	[91]
Amino acids (PTH-Ala, Leu, NorLeu, IsoLeu, Val, NorVal, Met, Phe, Tyr, Pro, Trp)	CZE	CM-β-CD	50 mM phosphate pH 6.5 and 10 mM CD	[92]
Methyl mandelate	CZE	CM-β-CD	50 mM phosphate pH 6.5 and 10 mM CD	[92]
Benzoin	CZE	CM-β-CD	50 mM phosphate pH 7 and 10 mM CD	[92]
Oxprenolol	CZE	CM-β-CD (DS 2–8)	100 mM borate buffer pH 8.2 and 0.625% CD (no baseline separation)	[97]
Aminomethyl-benzodioxane derivative (AMEBD)	CZE	CM-β-CD (DS 2-8)	100 mM borate buffer pH 8.2 and 0.625% CD	[97]

Table 4. Continued

Compound	CE type	CD	BGE	References
Thalidomide and hydroxy-thalidomite	CZE	CM-β-CD	50 mM phosphate buffer pH 6 and 15 mM CD	[93]
1,1′-Bi-2-naphthol 1,1′-Binaphthyl-2,2′-diamine	CZE	CM-β-CD	50 mM phosphate buffer pH 6 and 0.5 mM CD	[28]
Ephedrine	CZE	CM-β-CD (DS 2–8)	100 mM borate buffer pH 8.2 and 0.625% CD (no baseline separation)	[97]
Dihydropyridine calcium antagonists (nisoldipine, nimodipine, nitredipine, isradipine, amlodipine)	CZE or CD-MEKC	CM-β-CD	20 mM phosphate-borate pH 4.6 or 6.7 or 7-urea 0.5–1% and 5–10 mM CD (10 mM buffer pH 6.7, 0.5% urea, 20 mM SDS and 5 mM C.S. for CD-MEKC)	[185]
	CZE	CM-β-CD (mM)	50 mM phosphate buffer pH	[29]
Benzoin		5	6	
Methylether-2,2′-dihydroxy-1,1-binaphthyl		5	6	
5-methyl-5-phenylhydantoin		5	4.5	
Thalidomide		5	4.5	
	CZE	CM-γ-CD (mM)	50 mM phosphate buffer pH 5	[92]
Epinastine		0.1		
Primaquine		0.1		
Promethazine		0.1		
Imazalil		0.5		
Aminoglutethimide		1		
Azelastine		1		
Chlorpheniramine		1		
Eperisone		1		
Nicardipine		1		
Tolperisone		1		
Verapamil		1		
Clorprenaline		2		
Meclizine		3		
Thioridazine		5		
trimipramine		5		
Pindolol		5		
Trimebutine		5		
Bupivacaine		10		
Sulconazole		10		
Trimetoquinol		10		
Benzoin	CZE	CM-β-CD or β-CD-phosphate	50 mM phosphate buffer pH 7 and 10 mM CD	[92]
Mandelate (methyl-, benzyl-, ethyl-mandelate)	CZE	CM-β-CD or β-CD-phosphate or SBE-βCD	50 mM phosphate buffer pH 6.5-7 and 10 mM CD	[92]

Table 4. Continued

Compound	CE type	CD	BGE	References
Amino acids [6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC)-Arg, Lys, His]	CZE	CM-β-CD polymer	10 m*M* borate buffer (BTP) pH 7 and 5 m*M* CD	[61]
Benzoin Methyl ether benzoin Hydrobenzoin Chlorthalidon Mandelic acid *p*-Hydroxy mandelic acid *m*-Hydroxy mandelic acid Atrolactic acid Phenyllactic acid Phenylpropionic acid Phenylbutyric acid	CZE	Mono(6-amino-6-deoxy)-β-CD	phosphoric acid–ammediol buffer pH 2.3 (ionic strength 24 m*M*) and 0–20 m*M* CD	[186]
Aryl propionic acids (carprofen, flurbiprofen, ketoprofen, naproxen, suprofen) Benzoin, methylbenzoin	CZE	Mono(6-amino-6-deoxy)-β-CD/TM-β-CD	phosphoric acid–ammediol buffer pH 2.3 (ionic strength 24 m*M*) and 20 m*M* CD-NH2＋0–100 m*M* TM-CD	[187]
Atenolol	CZE	SBE-β-CD	Litium phosphate buffer pH 2.2 and 1.5 m*M* CD	[52]
Methyl mandelate Ethyl mandelate 1-Phenylethylalcohol	CZE	SBE-β-CD	50 m*M* phosphate pH 6 and 10 m*M* CD	[92]
1-Acenaphthelol	CZE	SBE-β-CD	50 m*M* phosphate pH 7 and 10 m*M* CD	[92]
Amino acids (PTH-Ala, Leu, Norleu, Isoleu, Val, Norval, Met, aminobutyrric ac., Phe, Tyr, Pro, Trp)	CZE	SBE-β-CD	50 m*M* phosphate buffer pH 6.5 and 10 m*M* CD	[92]
Alcohols (2-phenyl-2-butanol, 1-phenylethanol, α-ethylphenethyl alcohol, 1-phenyl-butanol, 1-phenyl-1,2-ethanediol)	CZE	SBE-β-CD	50 m*M* phosphate pH 6 or 50 m*M* tris–HCl pH 8 or 50 m*M* borate pH 9 and different amount of CD	[88]
Amino acids (Dansyl-Phe, Trp, Met, norVal, Leu)	CZE	SBE-β-CD	50 m*M* phosphate pH 8 and 3–20 mg/ml of CD	[88]
Acenocoumarol Atenolol Bupivacaine Metoprolol Oxprenolol Pindolol Promethazine	CZE	SBE-β-CD	50 m*M* phosphate pH 6 and 1–20 mg/ml of CD	[88]

Table 4. Continued

Compound	CE type	CD	BGE	References
Propranolol				
Terbutaline				
Warfarin				
Tetracyclic indol derivative	CZE	SBE-γ-CD	40 mM phosphate pH 3 and 1 mM CD-SBE	[99]
1,1′-Bi-2-naphthol 1,1′-Binaphthyl-2,2′-diamine	CZE	SBE-β-CD	50 mM phosphate buffer pH 6 and 0.5 mM CD	[28]
Herbicides (bromacil, chlorbufam, napropramide, ethofumesate, imazapir, flamprop isopropyl, flamprop free acid, haloxyfop free acid, fluazifop	CZE	SBE-β-CD	25 mM borate buffer pH 9 and 5–50 mg/ml of CD	[98]
	CZE	SBE-β-CD (mM)	50 mM phosphate buffer pH	[92]
Chlorpheniramine		0.2	5	
Clorprenaline		0.2	5	
Epinastine		0.2	5	
Terbutaline		0.2	5	
Trimetoquinol		0.2	5	
Denopamine		0.5	5	
Nicardipine		0.5	5	
Etilefrin		1	5	
Phenylephrine		1	5	
Sulpiride		1	5	
Atenolol		2	5	
Mexiletine		2	5	
Trimebutine		2	5	
	CZE	SBE-β-CD (mM)	50 mM phosphate buffer pH	[92]
Trihexyphenidyl		2	5	
Aminoglutethimide		3	5	
Verapamil		3	5	
Chlormezanone		5	5	
Eperisone		5	5	
Fenoterol		5	5	
Imazalil		5	5	
Oxyphencyclimine		5	5	
Meclizine		5	5	
Primaquine		5	5	
Promethazine		5	5	
Sulconazole		5	5	
Trimipramine		5	5	
	CZE	SBE-β-CD (mM)	50 mM phosphate buffer pH	[92]
Acebutolol		3	5	
Bunitrolol		3	5	
Bupivacaine		3	5	
Salbutamol		3	5	
	CZE	SBE-β-CD [mM]	50 mM phosphate buffer pH	[29]
Metomidate		1	9	
Benzoin		2	6	

Table 4. Continued

Compound	CE type	CD	BGE	References
Enilconazole		1/10% methanol	9	
5-Methyl-5-phenylhydantoin		2	4.5	
Thalidomide		2	4.5	
Hexobarbital		2	9	
1-Methyl-5-ethyl-5-propylbarbital		5	6	
Mianserine		0.5	9	
Mianserine (MN) and its analogues 7-CH3 -Cl 8-CH3 -OCH3 -Cl -F 9-CH3 10 trans-OH 12-Cl 13-CH3	CZE	SBE-β-CD 0.1 mM or SEE-β-CD 0.1 mM or CM-β-CD 5 mM	50 mM phosphate buffer pH 3.0	[47]
Dansyl-amino acids (DNS-Trp, Phe, norLeu, Leu, Met, norVal, Glu, Asp)	CZE	SBE-β-CD 1%	10 mM phosphate buffer pH 3.1	[48]
Dipeptides (DL-Leu-DL-Tyr)	CZE	1% SBE-β-CD	10 mM phosphate buffer pH 3.1 or 6.5	
Chlorophenols (2,3,5-TCP; 2,4,5-TCP; 2,4,6-TCP; 2,3,6-TCP; 2,5-TCP; 4-CP; 3-CP; 2,6-DCP; 2-CP; phenol)	CZE	SBE-β-CD 1%	10 mM phosphate buffer pH 3.1 or 6.5	
Sulindac, fenoprofen, ketoprofen, warfarin, hexobarbital	CZE	SBE-β-CD and DM-β-CD	100 mM phosphoric acid–triethanolamine pH 3 and 5 and 10 mM of charged and uncharged CD, respectively	[101]
Mianserine	CZE	SBE-γ-CD	40 mM phosphate pH 3 and 0.04 mM CD-SBE	[99]
1,1′-Bi-2-naphthol 1,1′-Binaphthyl-2,2′-diamine	CZE	SEE-β-CD	50 mM phosphate buffer pH 6 and 0.5 mM CD	[28]
5-Methyl-5-phenylhydantoin	CZE	SEE-β-CD (mM) 2	50 mM phosphate buffer pH 4.5	[29]
Thalidomide		2	4.5	
Trimipramine	CZE	Sulfated-β-CD	150 mM citric acid-100 mM Tris pH* 5.1 and 10 mM CD in formamide	[90]
α-Pinene β-Pinene Camphene Limonene Tetralin Terbutaline	CZE	Sulfated-β-CD /α-CD	10 mm phosphate buffer pH 3.3 and 1.3 or 6.5 mM of sulfated-CD	[188]

Table 5
Enantiomeric separations with sulfated cyclodextrins

Compounds	R	Compounds	R	Compounds	R
5-(4-Methylphenyl)-5-phenyl-hydantoin	5.4	*Anthihistamines*		*Antidepressants*	
5-(4-Hydroxyphenyl)-5-phenyl-hydantoin	5.6	Chlorpheniramine	2.3	Bupropion	1.6
5-Chlorobutyl-5-5-Phenyl-hydantoin	26.0	Carbinoxamine	3.2	Trimipramine	1.1
Phensuximide	8.3	Doxylamine	0.6	Tranylcypromine	2.4
Benzoin	2.8	Dimethindene	4.1	Nefopam	1.1
Hydrobenzoin	26.0	*Antiarrhytmics*		*Miscellaneous*	
9-Methyl-$\Delta^{5,10}$-octalin-1,6-dione	6.5	Verapamil	5.0	Aminogluthetimide	5.8
1,1′-Binaphthyl-2,2′-diol	11.1	Mexiletine	3.6	Canadine	3.4
1,1′-Binaphthyl-2,2′-dihydrogen phosphate	2.4	Disopyramide	7.4	Idazoxan	2.2
1,2,3,4-tetrahydro-1-naphtol	2.2	*Anticholinergics*		Isoxsuprine	1.0
1-(3-Chlorophenyl)-ethanol	4.9	Trihexylphenidyl	1.3	Laudanosine	8.1
α-Cyclopropylbenzyl alcohol	15.7	Oxyphencyclimine	0.8	Tetrahydropapaveroline	5.6
2,3-Dimethyl-1-phenyl-1-propanol	2.6	Mepenzolate	1.7	Methoxyphenamine	12.6
1,2-Diphenyl-2-propanol	2.7	*β-blockers*		Midodrine	4.6
Warfarin	8.6	Piperoxan	6.1	*Miscellaneous*	
Anaesthetics		Pindolol	2.3	Orphenadrine	1.5
Ketamine	3.0	Alprenolol	4.0	Terbutaline	3.8
Mepivacaine	4.0	Oxprenolol	8.4	Tetramisole	3.8
Bupivacaine	2.6	Acebutolol	1.9	Tolperisone	3.4
Anthihistamines		*Antimalarials*		Troger's base	16.1
Pheniramine	1.0	Chloroquine	2.6		
Bromopheniramine	1.9	Hydroxychloroquine	3.8		

10 m*M* phosphate buffer pH 3.8 and 2% sulphated-β-CD (7–10 DS) (from Ref. [189]).
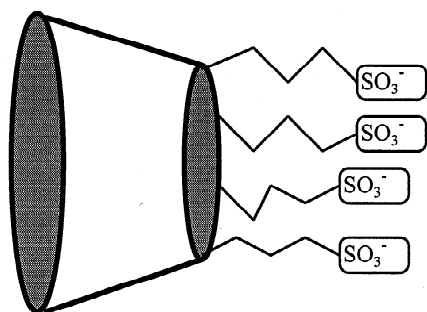

Fig. 10. Structure of sulfobutylated-β-cyclodextrin.

using inclusion–complexation is reversing the migration order of two enantiomers; this is, very often, requested in analytical chemistry when purity control of a drug, where the mixture contains an enantiomeric excess, has to be performed. It is well known that when the impurity moves behind its isomer in excess, the resolution can be reduced or even lost, however peak integration is not easy. Thus in CE, in order to reverse the migration order of two enantiomers, several approaches can be used, e.g., reversing the eof by using cationic additives and selecting different CDs, especially charged ones.

Schmitt and Engelhardt [83] reversed the migration order of hexobarbital and DNS-Phe adding to

the BGE at pH 8.3, with HP-β-CD, spermidine or spermine. A similar effect was obtained using CM-β-CD instead of DM-β-CD for the enantiomeric separation of ephedrine.

Binaphthyldihyl hydrogenphosphate has been resolved in its enantiomers at pH 6 using SBE-β-CD or CM-β-CD; the analysis has been performed in the presence of the eof and thus the analytes moved in the direction of the cathode (behind the eof). Changing the pH to 3.7, the eof was very low and the sample was injected at the cathodic end and the migration order was reversed only with the sulfobutylated CD (S eluted first). NMR studies revealed that the complex of all CDs used was stronger with the $S(+)$-enantiomer than with the $R(-)$ one and it was concluded that the inversion of migration order was due to the self mobility of SBE-β-CD (higher than those of analytes). Experiments performed at pH 3, 4.3 and 5 using CM-β-CD showed no resolution of analytes at pH 4.3 and the migration order was reversed at pH 5 [$S(+)$ faster than $R(-)$] [89].

From the examples given above and the list reported at the end of this review, we can outline that negatively-charged CDs are powerful chiral selectors, able to separate a wide number of racemic analytes into their enantiomers.

4.1.3. Effect of the pH of the background electrolyte, concentration of the buffer and its ionic strength, type of counter-ion

The type of counter-ion, the ionic strength and the concentration of the BGE are very important parameters to be controlled in chiral analysis by CE. In fact the analyte hydrophobicity can be modified by the counter-ion and thus the inclusion–complexation influenced.

The effect of the counter-ion, present in the BGE, has been studied by both CZE and ITP [102,103].

The increase of ionic strength and/or concentration of the buffer also influences the eof and thus the migration time. However, higher ionic strength is responsible for the increase in current that can cause a reduction in efficiency. Peak tailing has been observed by Altria et al. [104] decreasing the ionic strength and this negative effect (electromigration dispersion) can be reduced using a concentration of

the BGE 100 times higher than that of analyte. Williams and Vigh studied the enantiomeric separation of several (α-hydroxymethylbenzyl)trialkyl ammonium ions using β-CD and demonstrated the power of proper co-ion mobility matching [105].

The effect of buffer concentration on resolution and sensitivity of isoproterenol enantiomers has been discussed by Hadwiger et al. [106]; a maximum of resolution, efficiency and sensitivity was found at 250 mM of sodium acetate, pH 4.75, containing 0.1 g/ml of methylated-β-CD. The optimized method was used for the analysis of microdialysis samples.

The theory proposed by Wren and Rowe [64,107,108] was based on an equilibrium model where the two diastereomeric complexes (analyte-CD) are assumed to have the same electrophoretic mobility. Interesting studies by Vigh's group proposed an extended model for the separation of enantiomers of weak electrolyte solutes considering both pH and concentration of chiral selector [109,110]. Desionoselective (type I), ionoselective (type II) and duoselective (type III) separation models have been proposed by the authors where only the non-dissociated forms, the dissociated forms and nondissociated forms of enantiomers, respectively, complex selectively. The duoselective model has been demonstrated for the separation of 3,5-dinitrobenzamido phenylalanine enantiomers calculating the association constants and ionic mobilities. Predicted peak resolution surfaces were compared with experimental data, however the authors concluded that the experimental data resolution results were lower than the theoretical ones due to the presence of electromigration dispersion [111].

The pH of the BGE is of paramount importance for enantiomeric separation by CE because its change may influence several parameters such as migration time, eof and resolution. This has been shown by several authors, e.g., Valko et al. [51] studied the enantiomeric separation of mandelic acid at pH 7–9 with different buffer type and γ-CD. The mobility of the enantiomers could be regulated by adjusting the pH of the electrolyte, the concentration of the CD and the temperature of the capillary.

Ren and Liu [112] discussed the effect of the pH on the enantiomeric resolution of dioxypromethazine enantiomers. The resolution decreased by increasing the pH in the range 2–7 and the authors accounted

for this effect by the increase in the charge and thus in the mobility of the enantiomers at low pH.

When using 18C6TA for the chiral separations by CE, the pH can play a very important role. In fact it has been shown that the enantiomeric resolution of some α-amino acids [113], studied at pH range of 2–4, decreased when the pH exceeded 3.0. The authors pointed out that the pH influenced the charge of both analytes and crown (pK are 4.88, 4.29, 2.84 and 2.13) and thus the crown derivatives were partly dissociated weakening the interaction with analytes.

4.1.4. Addition of organic solvent to the BGE

The separation of enantiomers by CE in non-aqueous media has been investigated using chiral selectors such as CDs or 18-crown-6-ether tetra-carboxylic acid. The organic solvent, added to the aqueous BGE can control the eof and can improve the selectivity of the separation. Another advantage on using organic solvents is the possibility of analysing compounds with low stability in water.

The use of 30% of methanol enabled the resolution of propranolol enantiomers by CZE employing β-CD in the presence of urea at pH 2.5 [68]. We also demonstrated that the enantiomeric resolution of flurbiprofen, an anti-inflammatory drug, can be improved by adding 20% of methanol to the BGE at pH 5 containing 5 mM of TM-β-CD (without organic modifier, poor resolution was achieved).

As an example, Fig. 11 shows the effect of the concentration of methanol on the enantiomeric separation of flurbiprofen [114].

The effect of isopropanol on enantiomeric resolution using inclusion–complexation has been investigated by Wan et al. [115]. 2-(9-Antranyl)ethyl chloroformate (AEOC) amino acid derivatives were separated into their enantiomers using either β- or γ-CD dissolved in a phosphate buffer at pH 7.5 in the presence of SDS and different concentrations of isopropanol. An optimum concentration of organic modifier was found and the authors noted that the presence of isopropanol caused an inversion of migration order when using γ-CD, for amino acids having a non-polar side chain. In order to explain the resolution mechanism, it was proposed that the antrylic moiety or part of it, fits into the CD cavity and amino and carboxylic groups of the amino acid interact with the hydroxyl of the CD. The iso-
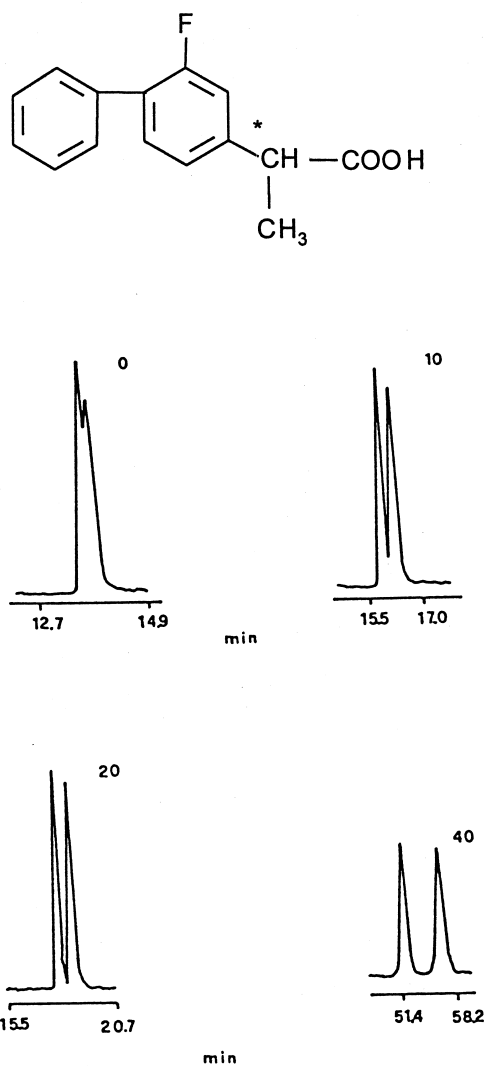

Fig. 11. Electrophoretic separation of flurbiprofen enantiomers using different concentrations of methanol as organic modifier of the background electrolyte. Capillary (polyacrylamide coated) 36 cm×0.05 mm I.D.; run buffer, 100 mM MES pH 5 and 5 mM TM-β-CD and methanol; applied voltage, 20 kV (modified from Ref. [114]).

propanol influenced the interactions between CD-analytes and SDS.

Improvement in resolution of enantiomeric separation, using an aqueous–organic buffer, has also been shown by other authors using CDs as chiral selectors [35,114–120]. Besides the improved resolution achieved by adding the BGE organic modifiers, we have to remark that in some instances the

enantioselectivity can be reduced [121]. This effect can be explained considering the theory discussed by Wren [122]; when CD was at or below the optimum concentration (maximum of resolution), the addition of organic modifier caused a decrease in resolution due to the change of association constants of the analyte-CD.

In a recent publication, Mori et al. [123] studied the enantiomeric resolution of aromatic amines, amino acids and several amino alcohols using 18C6H4 as the chiral selector dissolved in a non-aqueous media. The concentration of the chiral additive influenced the enantiomeric separation of the studied compounds which was improved by adding TBAP (tetra-*n*-butyl ammonium perchlorate) to the organic BGE (formamide). Excellent enantiomeric resolutions have been achieved and the authors ascribed these results to the interaction of the *n*-butyl group of TBAP with the substituent of the amino compounds. The substituent with TBAP ''behaves like a bulkier substituent than itself''. The organic solvent influences the stability constants of the host–guest enantiomeric complexes (the higher the dielectric constant of the solvent, the lower the stability constant is supposed). In the case of 1-phenylethylamine ε was 111 and 78 for formamide and water, respectively. The lower stability is responsible for the steric interaction between carboxylic groups of 18C6H4 and 1-phenylethylamine.

In a similar study, Wang and Khaledi [90] used β-CD, γ-CD and some β-CD derivatives dissolved in three organic solvents, namely formamide (FA), *N*-methylformamide (NMF) and *N,N*-dimethylformamide (DMF) and analyzed several pharmaceutical amines; the results have been compared with those obtained using the same CDs in water–urea buffer. The measured stability constants of trimipramine, mianserin, thioridazine with β-CD resulted to be $\sim10^4$ in water, ~10 in FA and $\sim10^{-2}$ in DMF. According to theoretical studies ($[CD]_{opt} = (1/\sqrt{K_1 K_2})$, [124]), the optimum CD was found at low concentration range in water–buffer (0.1 mM) and at relatively high concentration range (100 mM) in FA. For analytes binding strongly with CD, e.g., trimipramine or thioridazine, the $[CD]_{opt}$ was very low. The authors pointed out that in aqueous buffer, the inclusion–complexation of the two enantiomers into the CD cavity (hydrophobic interaction) is fun-

damental for the resolution mechanism and the hydrogen bonds with the hydroxyl groups on the CD rim provide secondary interactions (according with the three point rule). The same mechanism cannot be proposed when organic solvents are used, in fact the inclusion–complexation is very small or is not present at all. This interpretation of the separation mechanism is explained observing that in organic solvents tricyclic analytes, too large in order to fit β-CD, are resolved in this chiral selector (no resolution was achieved when γ-CD was employed). Thus it was supposed that the analytes are interacting with the hydroxyl groups on the CD rim (polar interactions).

The separation of dansyl-amino acid enantiomers in aqueous-phosphate-borate buffer and in *N*-methylformamide have been compared by Valko et al. [125] using β-CD. When the organic solvent was used, the β-CD concentration was relatively high (20–200 mM). The DNS-AA were moving behind the eof and in this BGE the repeatability for migration time of DNS-Ala was very good (R.S.D. 0.48–0.58%). The resolution increased by increasing the CD concentration.

4.1.5. Addition of polymers

Improvement of resolution, when inclusion–complexing agents are employed for enantiomer separations by CE, can be achieved by adding BGE polymers such as hydroxyethylcellulose (HEC), hydroxypropylmethylcellulose (HPMC), polyvinylalcohol (PVA).

As a result of the use of polymers, a reduction of eof and an increase of viscosity is obtained. Furthermore the adsorption of analytes on the capillary wall is avoided or reduced.

Strong eof should generally be avoided for a good enantiomeric resolution in CE because the time spent by the analyte for inclusion–complexation equilibria is reduced. This is obtained by performing the electrophoretic experiments at acidic pH (relatively low electroosmotic flow) and/or by using a coated capillary (e.g., polyacrylamide).

The usefulness of HEC or PVA additives has been shown for the improvement for enantiomeric resolution of racemic drugs such as threo-chloroamphenicol and ketotifen [126] and tocainide analogues [127]. Recently Nishi et al. [128] used a

phosphate buffer at pH 2.5 containing urea for the enantiomeric separation of denopamine enantiomers to study the effect of PVA, HPMC added to the BGE in uncoated or polyacrylamide-coated capillaries. The highest resolution of denopamine enantiomers was achieved when coated capillaries were used.

4.1.6. Addition of surfactants

The separation of uncharged enantiomers cannot be performed by CE using native CDs or modified uncharged CD unless an additional selective separation mechanism is introduced.

The solution to this analytical problem has been shown by Terabe's group adding CDs to a micellar solution; this technique is well known as Cyclodextrin-modified MEKC (CD-MEKC) [75]. In CD-MEKC, the CD molecules move in the electric field with the electroosmotic flow while the micellar phase moves with lower velocity due to its negative charge, (assuming no interactions between CD and micelles). The neutral compounds may interact either with the micelles and/or with the CD and will be resolved on the basis of these interactions.

Several papers document the applicability of the method showing the enantiomeric separation of: dansyl amino acids [60], tetrachloro-dibenzo-*p*-dioxin (TCDD) isomers [129], cicletanine enantiomers in plasma using γ-CD-SDS in the buffer [130], mephenytoin, phenytoin, 4-hydroxymephenytoin and 4-hydroxyphenytoin enantiomers were resolved using β-CD with SDS and were determined in urine samples [77], diniconazole, uniconazole and structurally-related compounds [131].

Recently several peptide enantiomers were separated by Wan and Blomberg [120], after derivatization with 9-fluorenylmethyl chloroformate (FMOC), by CD-MEKC using β- or γ-CD in the presence of SDS; the addition of organic solvent improved the enantiomeric resolution.

4.1.7. Mixed chiral selectors

Examples of chiral resolutions achieved using more than one separation mechanism have been reported, e.g., we separated Co(III) complexes by CE using both outer-sphere and inclusion–complexation modifying the BGE with L-tartrate and β-CD [132]. In a recent publication Horimai et al. succeeded in the enantiomeric separation of new drugs,

namely ofloxacin, DV-7751 and DU-6859 using ligand-exchange and inclusion–complexation. Here Zn(II)/D- or L-Phe was used in combination of γ-CD. The couple of enantiomers were not resolved in the absence of one of the chiral selectors. The resolution was strongly influenced by the cavity of the CD (only γ-CD allowed the separation); the most effective ligands were those with aromatic groups such as Phe or Tyr [133].

4.1.8. Effect of capillary temperature

Loss of enantiomeric resolution can be obtained when inclusion– complexing chiral selectors are used in CE. The increase of capillary temperature causes a decrease of the buffer viscosity and thus a decrease of the migration time of analytes. Furthermore, a change of this parameter, can strongly influence the stability of the inclusion complex formed during the electrophoretic run (usually the increase of temperature causes a decrease in the association constant. This effect has been shown for the enantiomeric separation of norepinephrine, epinephrine and isoproterenol using DM-β-CD where the best resolution of the studied enantiomers was achieved at 15°C [134]. The influence of capillary temperature on enantiomeric resolution and selectivity employing CDs as chiral selector has been recently reported [104,117,135,136]

4.1.9. Method optimization

Considering the wide number and CD type used in CE and the parameters influencing the enantioselectivity, a general rule to be followed for method optimization does not exist. However we would recommend the following steps:

(1) Consider the chemical structure of analytes in order to understand if it is charged or uncharged; this will advise the most useful BGE (concentration, pH etc.) and capillary type. This data can be obtained considering either the pK and/or electrophoretic experiments at different buffer pH. When basic compounds have to be analyzed, an acidic pH of the BGE can be selected and the enantiomeric separation can be performed either in a coated or uncoated capillary tube. The recommended buffers are phosphate or citrate at pH 2.5 and 3.5, respectively (50–100 mM). In some cases a pH lower (1 unit) than the pKa of the enantiomers allows the move-

ment of analytes as cations and a pH higher than 2.5–3.5 could be considered. In some case pHs$=$4–6 can be helpful for chiral separation.

In the case of acidic compounds, the general rules for basic compounds have to be considered. The selection of the pH of the BGE in the range 4.5–8 will charge (negatively) the two enantiomers and allow work with a sufficient eof responsible for the movement of the analytes toward the cathode. The use of the coated capillary allows work in a reversed mode and the inversion of migration can be obtained.

The analysis of uncharged enantiomers can be easily achieved by CE using a relatively strong eof, an uncharged CD in the presence of a charged chiral surfactant forming micelles (CD-MEKC) in untreated capillary. Alternatively, a charged CD can be employed, e.g., carboxymethylated or ethylated-β-cyclodextrin, sulfobutyl-ether-β-cyclodextrin or a charged β-cyclodextrin polymer (in either coated or untreated capillary).

(2) Observe the size and the substituent groups on the asymmetric carbon of the studied compounds in order to select the appropriate inclusion–complexation agent. If aromatic rings are not included in the chemical structure of analytes, α-cyclodextrin can be selected; the same choice can be made if the analyte contains only one aromatic ring unless substituents in the *ortho* or *meta* position are present (a reduction of complexation, caused by hindrance effect, can be obtained). When the compounds possess two aromatic rings (e.g. condensed) in their structure, certainly β-CD has to be selected while for more than two aromatic groups, γ-CD could be the appropriate chiral selector.

If the success of the enantiomeric separation cannot be achieved using native cyclodextrins, attention must be paid to the wide number of modified ones, e.g., dimethylated, trimethylated, hydroxypropylated etc. Furthermore charged cyclodextrins could be chosen selecting the appropriate electrophoretic mechanism and experimental parameters, e.g., the CD should generally possess the opposite charge of analytes.

As a general suggestion, the compound is run in the absence of CD's and the effective mobility calculated, then the run is performed with the same BGE containing a relatively low amount of CD (2.5–5 m*M*) in order to verify if the effective

mobility decreases. If the resolution is not obtained but a complexation is recorded, it is necessary to increase the concentration of the chiral selector until the success of the separation is obtained. In the case of β-CD the screening of the concentration is limited by its solubility ($<$20 m*M*) which can be increased by adding to the BGE urea (4–8 *M*) or methanol or ethanol ($<$30% v/v). Alternatively modified cyclodextrins can be selected for either improving the solubility and using different secondary stereoselective bonds. Optimize the CE method by finding the maximum of resolution in between the low–high CD concentration.

When compounds, containing in their chemical structures, primary amines, e.g., amino acids, peptides, have to be separated into their enantiomers, 18-crown-6-ether tetracarboxylic acid could be advantageously used. In this case, attention should be paid to the selection of the BGE (pH and cation type, the latter should not compete with analytes in the inclusion–complex mechanism).

5. Inclusion–complexation in capillary electrochromatography

The inclusion–complexation mechanism has also been used for the control of enantioselectivity with other capillary electrophoretic techniques such as electrochromatography. Here open tubular capillaries, with CD bound to the wall, have been employed [137–141] while Li and Lloyd separated uncharged and negatively-charged compounds in a packed capillary containing 5 μm β-CD-bonded silica particles [142]. Recently Lelievre et al. [143] used a chiral stationary phase (5 μm HP-β-CD-bonded silica particles) for the enantiomeric separation of chlorthalidone with a mobile phase composed of acetonitrile–5 m*M* phosphate buffer at pH 6.5. The best enantiomeric resolution of chlorthalidone was achieved at the lowest content of acetonitrile (40%).

6. Comparison CE and HPLC

Several authors compared the results obtained in CE and HPLC in order to understand the separation

mechanism of the enantiomeric separations using inclusion–complexation mechanism.

Denopamine enantiomers have been studied by both CE and HPLC by Nishi et al. [128]. In CE, the two optical isomers were completely separated using an acidic pH with DM-β-CD or acetyl-β-CD while employing TM-β-CD or HP-β-CD or β-CD polymer only partial resolution was achieved. In this case, the importance of the CD type on chiral recognition was clearly shown. Successful enantiomeric separation has been achieved by HPLC using a CSP, perphenylated-β-CD column. The authors supposed an inclusion–complexation mechanism where the presence of hydrophobic groups (acetyl or methyl) were very important. Their observations were supported by the experiments performed in HPLC where phenyl groups were attached to the mouths of the CD. Denopamine was not resolved into its enantiomers when a CSP containing native β-CD was used.

Piperaki et al. [53] carried out a systematic approach to enantiomeric separation of fluoxetine and norfluoxetine using CE and HPLC. CD was added to the BGE or in the mobile phase (HPLC) or was bonded to the stationary phase. The association constants analyte-CD were calculated with both techniques and were in agreement; the authors concluded that by studying the influence of organic solvent content, CE can be useful to predict the experimental conditions for HPLC separation when using a CSP containing CD.

Gahm and Stalcup [54] compared the results obtained in CE with those of HPLC using a new CD (NEC-β-CD) for the enantiomeric separation of amino acid derivatives

Nishi et al. studied the enantiomeric separation of several primary amines by CE using charged and uncharged crown ethers. Thirteen of the seventeen couples of enantiomers were resolved with 18-crow-n-6-ether tetracarboxylic acid at low pH. The results achieved by CE were compared with those obtained in HPLC using a chiral crownpak CR(+) column. When using the chiral stationary phase in HPLC, a restriction of the usable organic solvent has been recognized [45].

7. Conclusions

CE is a powerful tool for the separation of chiral compounds using a wide number and type of enantioselective agents and among them, cyclodextrins or their derivatives, added to the background electrolyte, are the most widely used compounds. The main separation mechanism involved in the electrophoretic separation process is that of inclusion–complexation that also needs secondary stereoselective bonds between the analyte and the substituent groups on the CD rim. In CE, the use of other chiral compounds forming inclusion–complexation includes 18-crown-6-ether tetracarboxylic acid which is not as popular as CDs.

The selection of the appropriate inclusion–complexation agent and an optimization of other experimental parameters, e.g., concentration of the enantioselector, organic or polymeric additives, column temperature, counter-ion, pH and ionic strength of the BGE etc. are very important in order to optimize the chiral separation electrophoretic method. Theoretical approaches describing the enantiomeric resolution mechanism should be carefully considered in order to find the appropriate operational parameters.

8. Abbreviations

2,3-diac-β-CD	2,3-Diacetylated-β-cyclodextrin
8C6TA	18-Crown-6-ether-tetracarboxylic acid
AC-β-CD	Acetylated-β-cyclodextrin
AEOC	2-(9-Antranyl)ethylchloroformate
BGE	Background electrolyte
CD	Cyclodextrin
CD-MEKC	Cyclodextrin-modified micellar electrokinetic chromatography
CE	Capillary electrophoresis
CGE	Capillary gel electrophoresis
CZE	Capillary zone electrophoresis
EC	Electrochromatography
eof	Electroosmotic flow
CHES	2-(N-Cyclohexylamino)ethane sulphonic acid
CM-β-CD	Carboxymethylated-β-cyclodextrin
C.S.	Chiral selector
DM-β-CD	Heptakis(2,6-di-O-methyl)-β-cyclodextrin
DMF	Dimethylformamide
Et-β-CD	Ethylated-β-cyclodextrin
FA	Formamide
FMOC	9-Fluoroethylmethylchloroformate
Glu-α-CD	6-O-α-D-glucosyl-α-cyclodextrin

HE-β-CD	Hydroxyethyl-β-cyclodextrin
HPAB	Hexadecylpropylmethylammonium bromide
HP-β-CD	Hydroxypropyl-β-cyclodextrin
HPLC	High-performance liquid chromatography
HPMC	Hydroxypropylmethyl cellulose
Mal-β-CD	6-*O*-α-D-maltosyl-β-cyclodextrin
MEKC	Micellar electrokinetic chromatography
NEC-β-CD	Naphthylethylcarbamoylated-β-cyclodextrin
NMF	*N*-Methylformamide
PTH	Phenyl thyohydantoin
TBAP	Tetra-*n*-butyl ammonium perchlorate
TM-β-CD	Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin
Tris	Tris(hydroxymethyl)amino methane
SBE-β-CD	Sulfobutylated-β-cyclodextrin
SC	Sodium cholate
SDC	Sodium deoxy cholate
SDS	Sodium dodecyl sulphate
TLC	Thin layer chromatography
TMA	Tetra methylammonium
GC	Gas chromatography

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