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Review

Enantiomeric separations of drugs using mixtures of charged and neutral cyclodextrins

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Abstract

An overview on the use of mixtures of neutral and charged cyclodextrins as chiral additives for the enantioseparation of drugs by capillary electrophoresis is presented. These so called dual cyclodextrin systems can often provide unique selectivities. A brief theoretical background illustrating the influence of the chiral discrimination ability and the effective mobility of the two cyclodextrins on the overall selectivity of the enantiomeric separation is given. Typical examples of applications in the pharmaceutical field, based on the simultaneous use of a charged (cationic or anionic) and neutral cyclodextrins, are described.

Keywords: Reviews; Enantiomer separation; Background electrolyte composition; Cyclodextrins; Drugs

Contents

1. Introduction

Before a new chiral drug is approved, the pharmacological effects and metabolic pathways for each enantiomer must be studied [1]. Separation techniques have taken an essential role in all stages of the process because enantiomeric separation at an analytical scale is required for monitoring the enantiomeric purity of the chiral drug and for performing drug metabolism, pharmacokinetic and clinical studies.

Many papers dealing with applications of capillary electrophoresis (CE) to the enantioresolution of chiral compounds have been published over the past few years [2–4], as well as comprehensive reviews on chiral CE [5–12]. The most common approach for direct enantiomeric separation in CE involves the addition of cyclodextrins (CDs) to the running

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buffer. This is mainly due to the commercial availability of various kinds of native and derivatized CDs at relatively low cost, their good solubility in aqueous buffers, their UV transparency, their stability and their wide scope of application. The chemical modification of the native CDs leads to significant changes in their physicochemical properties and in their chiral recognition ability.

CD complexation can lead to enantiomeric resolution by CE provided that there are not only differences in stability between the complexes formed between the CD and the analyte enantiomers but also differences in mobility between the free and complexed forms of the analytes.

In contrast with neutral CDs, charged CD derivatives have their own electrophoretic mobility, which allows their use as carriers in electrokinetic chromatography for the separation of neutral or ionizable compounds in uncharged form [6].

In some cases CE enantioseparation cannot be achieved by addition of one chiral selector to the running buffer. However, the simultaneous use of a combination of two selectors can often lead to the enantioresolution of chiral compounds in CE. So far, several approaches have been explored.

Terabe et al. [13] and Lin et al. [14] used a combination of a neutral cyclodextrin (γCD and βCD, respectively) and a chiral micelle (sodium taurodeoxycholate) for the enantioseparation of Dns-DL-amino acids.

A mixture of a polymerized chiral micellar agent (poly(sodium *N*-undecylenyl-D-valinate) and γCD was also used successfully by Wang et al. to improve the enantioseparation of four enantiomeric pairs (laudanosine, 1,1′-binaphthyl-2,2′-diol, verapamil and 1,1′ binaphthyl-2,2′-diyl hydrogenphosphate) [15].

Another approach consists of using mixtures of two cyclodextrins (dual CD systems) to enhance the selectivity of the enantioseparation.

Neutral CDs have been employed in combination to separate the enantiomers of Dns-DL-amino acids (combination of β- and γCD) [13], binaphthyl compounds (combination of αCD and trimethyl-β-cyclodextrin: TMβCD) [16], phenoxy acid herbicides (combination of βCD and TMβCD or γCD and TMβCD) [17] and polychlorinated biphenyls (combination of β- and γCD) [18].

Recently, Fillet et al. [19] used in a pH 5.5 buffer a mixture of two charged CDs: carboxymethyl-β-cyclodextrin (CMβCD) and sulfobutyl-β-cyclodextrin (SBβCD), to separate the enantiomers of 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (BDHP). At this pH, both cyclodextrins are negatively charged and accelerate the analyte. Under these conditions, the combination of these two anionic cyclodextrins was found to be favourable to the enantioseparation. Previous studies have showed that CMβCD and SBβCD possess the same chiral recognition pattern, (+)-*S*-BDHP being more tightly bound by both cyclodextrins [20].

Jakubetz et al. [21] described systems in capillary electrochromatography (CEC) employing Chirasil-Dex (permethylated β-cyclodextrin covalently linked to dimethylpolysiloxane) as stationary phase and the anionic sulfo-*n*-propyl-βCD or the cationic 2-hydroxy-3-trimethylammoniumpropyl-βCD as mobile phase additive for the enantiomeric separation of hexobarbital.

The present review paper is more specially focussed on the addition of mixtures of charged and neutral cyclodextrins to the running buffer for the CE enantioseparation of drugs. The first application of this kind of dual systems has been described by Lurie et al. [22]. This topic has already been reviewed by Lurie in 1997 [10]. In the present paper, an update of CE applications based on the use of a combination of charged and neutral CDs is presented, as well as an overview of the different theoretical models developed in order to be able to explain and predict the changes in selectivity observed by use of this kind of systems.

2. Theoretical background

Until now, the nature and concentration of the individual CDs used in combination in dual systems has been largely empirical and in most cases the optimization of selectivity and resolution in such systems has been based on a trial and error approach.

$$\Delta\mu_{ep} = \mu_A - \mu_B = \frac{[C](\mu_f - \mu_c)(K_B - K_A)}{1 + [C](K_A + K_B) + K_A K_B [C]^2} \quad (1)$$

As clearly illustrated by Eq. (1) [23], the selectivity of the CE enantiomeric separation in the presence of a single chiral selector, which can be expressed by the mobility difference between the analyte enantiomers A and B ($\Delta\mu_{ep}$), depends not only on the chiral discrimination ability of the selector towards the enantiomers, represented by the difference between the two binding constants K_A and K_B, but also on the difference in electrophoretic mobility between the free and complexed forms of the analyte enantiomers ($\mu_f - \mu_c$).

Other equations have been developed to express the apparent electrophoretic mobilities of the analyte enantiomers, μ_A and μ_B, and the corresponding overall selectivity of the enantioseparation, usually represented either by the difference between μ_A and μ_B or by the ratio μ_A/μ_B [19,22–26] when two complexing agents, and in particular, cyclodextrins, are used in combination. All these equations are valid when only 1:1 complexation occurs between the analyte enantiomers and the CDs and the two CDs lead to independent complexation (absence of mixed complexes). Moreover, the two CDs are assumed to be pure, well characterized compounds, which is unfortunately rarely the case in practice, except for the native CDs, trimethyl-β-CD (TM-β-CD) and single-isomer substituted β-CD sulfates [12], and the electroosmotic flow is considered to be negligible.

Some of these equations have been simplified but they are only applicable to neutral solutes [24,25] or to dual CD systems in which one of two CDs is uncharged [22,25].

The following equation for the apparent electrophoretic mobility of the enantiomer A in dual CD systems (μ_A) is more general: it is valid for charged and neutral analyte enantiomers and for ionic and uncharged CDs:

$$\mu_A = \frac{1}{1 + K_{A.C_1}[C_1] + K_{A.C_2}[C_2]} \cdot (\mu_f + \mu_{c_1} K_{A.C_1}[C_1] + \mu_{c_2} K_{A.C_2}[C_2]) \quad (2)$$

where: $[C_1]$, $[C_2]$: concentrations of CD_1 and CD_2; μ_f: mobility of the analyte in free form; μ_{c_1}, μ_{c_2}: mobilities of the analyte–cyclodextrin complexes $A.C_1$ and $A.C_2$, respectively; $K_{A.C_1}$, $K_{A.C_2}$: equilibrium constants for the formation of complexes between enantiomer A and CD_1 or CD_2, respectively.

An analogous equation can be used to express the apparent electrophoretic mobility of the enantiomer B, μ_B. Using this kind of equations, the changes in selectivity obtained in dual CD systems can usually be predicted and the optimisation of these systems with respect to the migration times and the separation of the analyte enantiomers is facilitated.

It is obvious that in some dual systems, the chiral discrimination ability of one of the CDs can only lead to enantioseparation because of the presence of the second CD. For example, enantiomers of uncharged chiral analytes can be recognized stereoselectively by a neutral CD but no enantiomeric separation can be obtained using this neutral CD alone because of the lack of mobility difference between the free and complexed analyte enantiomers ($\mu_f = \mu_c$ in Eq. (1). When this neutral CD is used in combination with a charged CD, the latter can provide an apparent electrophoretic mobility to the analyte, which permits the enantioseparation. Under these conditions, it is not necessary for the charged CD to be enantioselective. Such a dual CD system employing a highly selective non-ionic CD in combination with a non-selective or poorly selective charged CD is always beneficial, since a complete enantioresolution of neutral solutes can only be achieved if the two CDs are used simultaneously. Moreover, it is not necessary in principle to know the affinity pattern of the analyte enantiomers for the highly selective CD since the other CD possesses no or poor enantioselectivity towards the analyte.

Such a situation is illustrated by the electropherograms presented in Fig. 1. The non-steroidal anti-inflammatory drug ketoprofen which is essentially in uncharged form at pH 3 was only partly enantioseparated with the heptakis-6-sulfato-β-cyclodextrin (HSβCD) and no enantiomeric resolution was observed with the neutral trimethyl-β-CD (TMβCD). By contrast, high chiral resolution was obtained by use of a dual system containing these two CDs, clearly indicating that TMβCD was in fact highly enantioselective towards ketoprofen in uncharged form.

Similar effects as those observed by the addition of a charged CD to a CE buffer containing a highly enantioselective neutral CD towards uncharged sol-

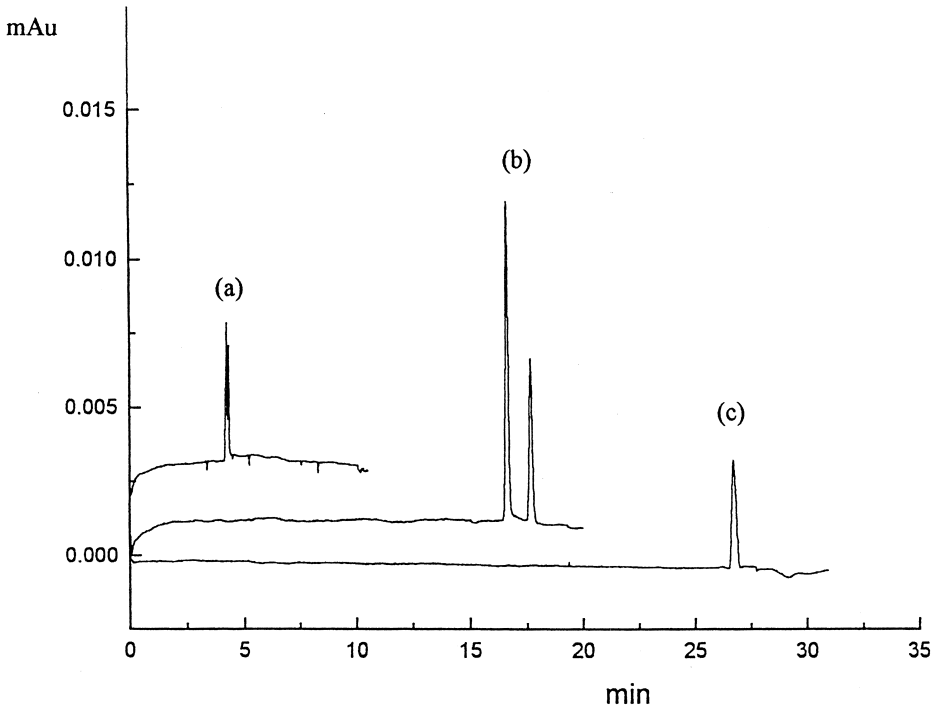

Fig. 1. Enantioseparation of ketoprofen Buffer: 100 m*M* phosphate buffer (pH3.0) containing: (a) 2.5 m*M* HSβCD; (b) 2.5 m*M* HSβCD and 30 m*M* TMβCD; (c) 30 m*M* TMβCD. Capillary: bare fused-silica, 44 cm (37 cm to the detector window) ×50 μm I.D.; temperature: 25°C, voltage: −25 kV; detection wavelength: 210 nm, hydrodynamic injection: 6 s. From Ref. [36].

utes can be obtained by using other secondary equilibria which can also generate a mobility difference between the analyte enantiomers and their complexes with the neutral CD, such as the addition of an achiral surfactant like SDS to create a MEKC system, the addition of borate ions to carbohydrates to form negatively charged complexes or a change of the buffer pH in the case of ionizable compounds [24–26].

From expressions such as Eq. 2, it can also be deduced that in dual CD systems in which the two CDs are enantioselective and have opposite effects on the migration of the analyte enantiomers, i.e. one CD accelerates them while the other one decelerates them, an improvement in selectivity will only be obtained if the affinity pattern of the enantiomers for each CD is opposite. The same requirement must be fulfilled if one of the two selective CDs has no effect on the analyte mobility, such as a neutral CD towards an uncharged compound. Similar deductions have been made by several authors [24–27] and the validity of these deductions was demonstrated experimentally [19,24,25].

As illustrated in Fig. 2, a detrimental effect on selectivity and resolution can be obtained in such dual CD systems if the affinity pattern is the same for the two CDs [19,26]. All CE separations presented in Fig. 2 were performed in the reversed polarity mode. At pH 2, the anionic sulfobutyl-β-CD (SBβCD) accelerates the enantiomers of BDHP, while the uncharged carboxymethyl-β-CD (CMβCD) decelerates them. However, SBβCD and CMβCD possess the same chiral recognition pattern, *S*-(+)-BDHP being more strongly bound by both of them. Consequently, the simultaneous addition of SBβCD and CMβCD leads to a loss of the separation selectivity (Fig. 2d), while a complete enantiomeric resolution was obtained in single CD systems with either SBβCD or CMβCD (Figs. 2b and c).

Dual CD systems in which the two CDs exhibit the same effect on the analyte mobility (both CDs either accelerate or decelerate the analyte enantio-

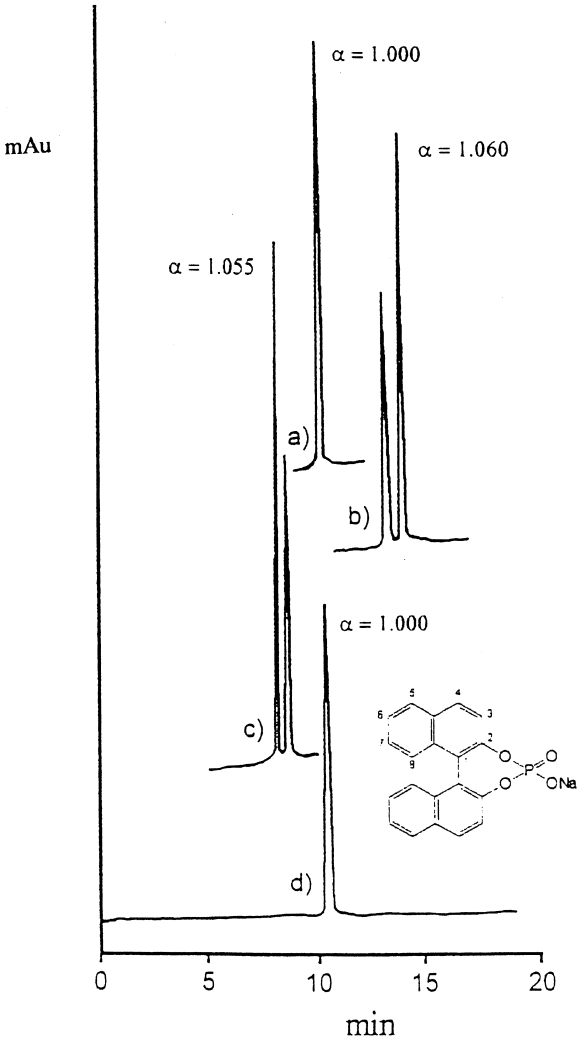

Fig. 2. Enantioseparation of a non-racemic mixture [(+)/(−)=2/1] of BDHP Buffers: 100 m*M* phosphoric acid/triethanolamine (pH 2.0) containing (a) no CD (b) 5 mg ml^{-1} CMβCD (c) 5 mg ml^{-1} SBβCD (d) 5 mg ml^{-1} CMβCD+5 mg ml^{-1} SBβCD. Capillary: bare fused-silica, 60 cm (43 cm to the detector window) ×50 μm I.D.; temperature: 23°C, voltage: −25 kV; detection wavelength: 210 nm. From Ref. [19].

mers, like two uncharged CDs towards charged solutes), seem to be in principle of more limited interest. It is not obvious that the combined use of such CDs can lead to a significant improvement of the selectivity of the enantioseparation. Expressions such as Eq. (2) seem to indicate that in such cases, the highest selectivity would be generally obtained by use of the more selective CD alone at its optimal concentration. However, this optimal concentration may be sometimes rather high, making a dual CD system more attractive. Such dual CD systems can also be useful in achiral separations [10]

On the other hand, it is not always possible to explain the changes in chiral selectivity observed in dual CD systems. In some cases, synergistic or antagonistic effects on chiral recognition can occur between the two CDs used in combination, which makes the overall effect on selectivity quite unpredictable [19].

Besides CE, other instrumental techniques such as NMR spectroscopy or mass spectrometry can be used to obtain complementary information about stoichiometry, binding constants and stereoselectivity in intermolecular analyte–selector interactions [26].

^{1}H and ^{13}C NMR, homonuclear correlated spectroscopy, heteronuclear chemical shift correlation and one dimensional rotating frame nuclear Overhauser enhancement provides information on the chiral recognition mechanism at a molecular level. Good correlation between data obtained in CE and NMR can be expected since the solute– selector interactions take place in a similar or even identical environment.

The stoichiometry of the solute– selector complexes can also be determined by mass spectrometry (ESI-MS–CE). The latter technique can detect the ions with m/z ratios corresponding to complexes with different stoichiometry but the results obtained by this method should be interpreted carefully because it is difficult to avoid the formation of false adducts.

X-ray crystallography can also be useful for studying the structures of the solute–selector complexes. However, structural data in the solid state should be transferred carefully to the structure of complexes in solution.

3. Dual systems using mixtures of cationic and neutral cyclodextrins

A baseline resolution of several arylpropionic acids (carprofen, flurbiprofen, ketoprofen, naproxen and suprofen) was obtained by Lelièvre et al. using a pH 2.3 buffer containing 20 m*M* 6-monoamino-6-

deoxy-β-cyclodextrin (NH_2βCD) at 20 m*M* concentration and TMβCD at different concentrations (cf. Table 1) [24].

The use of the neutral TMβCD alone did not lead to any enantioseparation since the solutes were completely protonated at this pH. By using the cationic NH_2βCD alone in the background electrolyte (BGE), no enantioseparation was observed either. However, complexation between the analytes and the cationic cyclodextrin occurred since the effective mobility of the arylpropionic acids increased with increasing concentration of NH_2βCD.

The combination of these two CD derivatives was then tested and enantiomeric separations were observed. In these dual systems, the positively charged CD provided the differential migration which led to the enantioseparation while the neutral cyclodextrin brought about enantioselectivity. The TMβCD concentration was optimized and quite different optimal concentrations were obtained for the arylpropionic acids studied (cf. Table 1). A electropherogram illustrating the separation of naproxen enantiomers using the dual system NH_2βCD/TMβCD (20/10 m*M*) is presented in Fig. 3.

The enantioseparation of these acidic drugs at pH 2.3 confirms the fact that selectivity originates from the complexation between the uncharged forms of these drug enantiomers and the neutral cyclodextrin.

The use of such dual CD systems was found to be an interesting alternative for the enantioseparation of neutral drugs.

In the same paper [24], Lelièvre et al. also developed a theoretical model for predicting selectivity in enantiomeric separations that involve two complexing agents. Binding constants for the NH_2βCD – analyte complexes were first evaluated. Then, apparent constants for the complexation between analyte enantiomers and TMβCD in the presence of 20 m*M* NH_2βCD at pH 2.3 were determined and the concept of intrinsic selectivity of a complexing agent Y in the presence of a given concentration of a complexing agent X was introduced [24].

4. Dual systems using mixtures of anionic and neutral cyclodextrins

Lurie et al. [22] demonstrated that the use of mixtures of the neutral dimethyl-β-cyclodextrin (DMβCD) and the anionic sulfobutyl-β-cyclodextrin (SBβCD) was suitable for the analysis of a number of basic chiral drugs of forensic interest. The presence of DMβCD (5 m*M*) alone in the BGE (25 m*M* Tris-phosphate of pH 2.4 containing 1.2% methanol) led to the enantioseparation of secondary amines like ephedrine, pseudoephedrine, methcathinone and methamphetamine and primary amines such as norpseudoephedrine and norephedrine, but failed to completely separate amphetamine and the tertiary amine propoxyphene.

However, with a mixture of DMβCD (5 m*M*) and SBβCD (1 m*M*) amphetamine and propoxyphene were completely resolved and the selectivity of most enantioseparations was increased.

Peak shapes were improved and shorter analysis times were obtained using 0.5 m*M* SBβCD instead of 1 m*M* SBβCD while keeping DMβCD at 5 m*M*, the mobility mismatch between the analytes and the cationic component of the BGE being then reduced.

Chankvetadze [11] interpreted these results as follows: DMβCD and SBβCD both decelerate the analyte and therefore a gain in the separation selectivity may be observed for the analytes which possess the same affinity pattern for both chiral selectors (pseudoephedrine). In contrast, the same combination of CDs will be unfavourable for the separation of enantiomers for which the cyclodextrins exhibit an opposite recognition pattern (cathinone, methcathinone and ephedrine).

Anigbogu et al. [28] reported the use of a dual cyclodextrin system for the resolution of aminoglutethimide. At pH 9, aminoglutethimide is present in uncharged form and its enantiomers could be completely resolved using a running buffer containing a 5 m*M* concentration of the anionic carboxymethyl-β-cyclodextrin (CMβCD), a 1 m*M* concentration of β-CD and 50% of methanol to the BGE leads to an expansion of the migration window.

The use of βCD alone could not provide enantiomeric resolution for this solute since aminoglutethimide in both free and complexed forms migrated under these conditions with the electroosmotic flow (EOF). Furthermore, despite of an obvious complexation between CMβCD and aminoglutethimide, no chiral resolution was observed with this CD derivative. However, when the neutral β-cyclodextrin was added to a running buffer con-

Table 1
Conditions for the enantioseparation of drugs using dual cyclodextrin systems[a]

Analyte	Type of dual CD system	Concentration of the CDs	BGE	R_s	α	Ref.
Adrenaline	SO_4βCD/αCD	10/20	A	3.4		[35]
	SO_4βCD/βCD	10/10	A	3.7		[35]
	SO_4βCD/γCD	10/20	A	2.9		[35]
	SO_4βCD/HPαCD	10/2%	A	2.5		[35]
	SO_4βCD/HPβCD	10/1%	A	3.4		[35]
	SO_4βCD/HPγCD	10/2%	A	3.6		[35]
Aminoglutethimide	CMβCD/βCD	5/1	B	1.4		[28]
Amphetamine	SBβCD/DMβCD	1/5	C	2.2		[23]
	SBβCD/DMβCD	0.5/5	C	1.3		[23]
Benzoin	NH_2βCD /TMβCD≅	20/122	D	1.2	1.100	[24,26]
Benzoin methyl ether	NH_2βCD /TMβCD≅	20/100	D	1.1	1.100	[24,26]
Brompheniramine	CMβCD/βCD	0.1/5 *	E	1.061		[19]
	CMβCD/TMβCD	0.1/10 *	E	1.045		[19]
Camphene	SO_4βCD/αCD	6.5/7.5	G	12		[29]
Carprofen	SBβCD/TMβCD	5/15	E	30.6		[30]
	NH_2βCD /TMβCD	20/10	D	1.35	1.130	[24,26]
Cathinone	SBβCD/DMβCD	1/5	C	0.5		[23]
	SBβCD/DMβCD	0.5/5	C	0.5		[23]
Chlormezanone	CMβCD/TMβCD	10/10	F	3.3		[31]
Chlorpheniramine	CMβCD/βCD	0.1/5 *	E	1.054		[19]
	CMβCD/TMβCD	0.1/5 *	E	1.031		[19]
	SO_4βCD/αCD	10/20	A	5.9		[35]
	SO_4βCD/βCD	10/10	A	3.2		[35]
	SO_4βCD/γCD	10/20	A	2.5		[35]
	SO_4βCD/HPαCD	10/2%	A	4.4		[35]
	SO_4βCD/HPβCD	10/1%	A	2.9		[35]
	SO_4βCD/HPγCD	10/2%	A	2.7		[35]
Chlorthalidone	CMβCD/TMβCD	10/10	E	2.4		[31]
Cocaine	SBβCD/DMβCD	1/5	C	3.8		[23]
	SBβCD/DMβCD	0.5/5	C	2.2		[23]
Denopamine	SO_4βCD/αCD	10/20	A	5.0		[35]
	SO_4βCD/βCD	10/10	A	4.6		[35]
	SO_4βCD/γCD	10/20	A	5.0		[35]
	SO_4βCD/HPαCD	10/2%	A	4.0		[35]
	SO_4βCD/HPβCD	10/1%	A	3.8		[35]
	SO_4βCD/HPγCD	10/2%	A	4.6		[35]
Dimethindene	CMβCD/βCD	0.5/5*	E	1.054		[19]
	CMβCD/TMβCD	0.5/10*	E	1.081		[19]
Ephedrine	CMβCD/βCD	2/15*	E	1.050		[19]
	CMβCD/DMβCD	2/15*	E	1.017		[19]
	SBβCD/DMβCD	1/5	C	1.0		[23]
	SBβCD/DMβCD	0.5/5	C	0.0		[23]
Fenoprofen	SBβCD/TMβCD	5/30	E	16.3		[30]
Flurbiprofen	SBβCD/TMβCD	5/30	E	16.3		[30]
	NH_2βCD /TMβCD	20/40	D	1.7	1.160	[24,26]
Hexobarbital	SBβCD/DMβCD	5/10	E	3.0		[27]
	CMβCD/DMβCD	10/10	F	3.2		[31]
Ibuprofen	SBβCD/TMβCD	5/30	E	5.6		[30]
	CMβCD/TMβCD	10/40	F	13.1		[33]
Indoprofen	SBβCD/TMβCD	5/40	E	4.9		[30]
	CMβCD/TMβCD	10/40	F	6.6		[33]
Ketoprofen	SBβCD/TMβCD	5/30	E	11.3		[30]
	NH_2βCD /TMβCD	20/80	D	2.1	1.210	[24,26]
Limonene	SO_4βCD/αCD	6.5/7.5	G	4		[29]
LY213829	SBβCD/βCD	10/7	H	> 1.5		[35]
Mephenitoin	SBβCD/TMβCD	5/10	E	5.2		[31]
Mephobarbital	SBβCD/TMβCD	5/40	E	3.9		[33]
	CMβCD/TMβCD	10/50	F	5.1		[31]

(*Continued on next page*)

Table 1 (*continued*)

Analyte	Type of dual CD system	Concentration of the CDs	BGE	R_s	α	Ref.
Methamphetamine	SBβCD/DMβCD	1/5	C	2.9		[23]
	SBβCD/DMβCD	0.5/5	C	1.6		[23]
Methcathinone	SBβCD/DMβCD	1/5	C	2.2		[23]
	SBβCD/DMβCD	0.5/5	C	0.8		[23]
Naproxen	SBβCD/TMβCD	5/20	E	5.4		[33]
	NH_2βCD /TMβCD	20/10	D	3.1	1.400	[24,26]
Noradrenaline	SO_4βCD/αCD	10/20	A	1.7		[35]
	SO_4βCD/βCD	10/10	A	1.2		[35]
	SO_4βCD/γCD	10/20	A	1.6		[35]
	SO_4βCD/HPαCD	10/2%	A	1.5		[35]
	SO_4βCD/HPβCD	10/1%	A	1.8		[35]
	SO_4βCD/HPγCD	10/2%	A	1.6		[35]
Norephedrine	SBβCD/DMβCD	1/5	C	1.0		[23]
	SBβCD/DMβCD	0.5/5	C	1.3		[23]
Norpseudoephedrine	SBβCD/DMβCD	1/5	C	6.4		[23]
	SBβCD/DMβCD	0.5/5	C	5.0		[23]
Pentobarbital	SBβCD/TMβCD	5/30	E	4.5		[33]
	CMβCD/TMβCD	10/50	F	7.7		[31]
Phenprocoumon	SBβCD/TMβCD	5/20	E	2.1		[33]
Propoxyphene	SBβCD/DMβCD	1/5	C	3.4		[23]
	SBβCD/DMβCD	0.5/5	C	2.0		[23]
Pseudoephedrine	SBβCD/DMβCD	1/5	C	8.7		[23]
	SBβCD/DMβCD	0.5/5	C	6.5		[23]
Secobarbital	SBβCD/TMβCD	5/30	E	1.9		[33]
	CMβCD/TMβCD	10/50	F	4.0		[31]
Sulfoxide metabolyte of LY213829	SBβCD/βCD	10/7	H	> 1.5		[35]
Sulindac	SBβCD/DMβCD	5/10	E	3.8		[30]
Suprofen	SBβCD/TMβCD	5/40	E	8.9		[30]
	NH_2βCD /TMβCD	20/40	D	1.6	1.140	[24,26]
Thiopental	SBβCD/TMβCD	5/40	E	2.7		[33]
	CMβCD/TMβCD	10/50	F	5.2		[31]
Tiaprofenic acid	SBβCD/TMβCD	5/40	E	7.3		[30]
Timepidum	SO_4βCD/αCD	10/20	A	1.7		[35]
	SO_4βCD/βCD	10/10	A	2.1		[35]
	SO_4βCD/γCD	10/20	A	0.9		[35]
	SO_4βCD/HPαCD	10/2%	A	1.7		[35]
	SO_4βCD/HPβCD	10/1%	A	2.4		[35]
	SO_4βCD/HPγCD	10/2%	A	1.5		[35]
Trimetoquinol	SO_4βCD/αCD	10/20	A	10.7		[35]
	SO_4βCD/βCD	10/10	A	10.7		[35]
	SO_4βCD/γCD	10/20	A	12.3		[35]
	SO_4βCD/HPαCD	10/2%	A	9.4		[35]
	SO_4βCD/HPβCD	10/1%	A	10.8		[35]
	SO_4βCD/HPγCD	10/2%	A	11.8		[35]
Verapamil	CMβCD/βCD	0.5/10 *	E	1.034		[19]
	CMβCD/TMβCD	0.5/5 *	E	1.070		[19]
Warfarin	SBβCD/TMβCD	5/40	E	12.9		[33]
α-Pinene	SO_4βCD/αCD	6.5/7.5	G	25		[29]
β-Pinene	SO_4βCD/αCD	6.5/7.5	G	12		[29]
1,1′-Bi-2-naphthol	PO_4βCD/αCD	10/10	I	1.1		[16]
1,1′-Binaphthyl-2,2′-dicarboxylic acid	PO_4βCD/αCD	10/10	I	2.7		[16]
1,1′-Binaphthyl-2,2′-diyl hydrogenphosphate	PO_4βCD/αCD	10/10	I	1.3		[16]
	PO_4βCD/TMβCD	5/20	I	2.2		

[a] The cyclodextrin concentrations are expressed in millimolar (m*M*), except for the examples with * for which the concentration is expressed in mg ml^{-1}. BGE: A: 100 m*M* phosphoric acid/triethylamine (pH 3); B: 10 m*M* phosphate/6 m*M* borate (pH 9), 50% MeOH; C: 25 m*M* Tris-phosphate (pH 2.4), 1.2% MeOH; D: 34 m*M* phosphate buffer (pH 2.3); E: 100 m*M* phosphoric acid/triethanolamine (pH 3); F: 100 m*M* phosphoric acid/triethanolamine (pH 5); G: 10 m*M* phosphate buffer (pH 3.3); H: 50 m*M* LiOH/phosphoric acid (pH 2.5); I: 25 m*M* phosphate (pH 9).

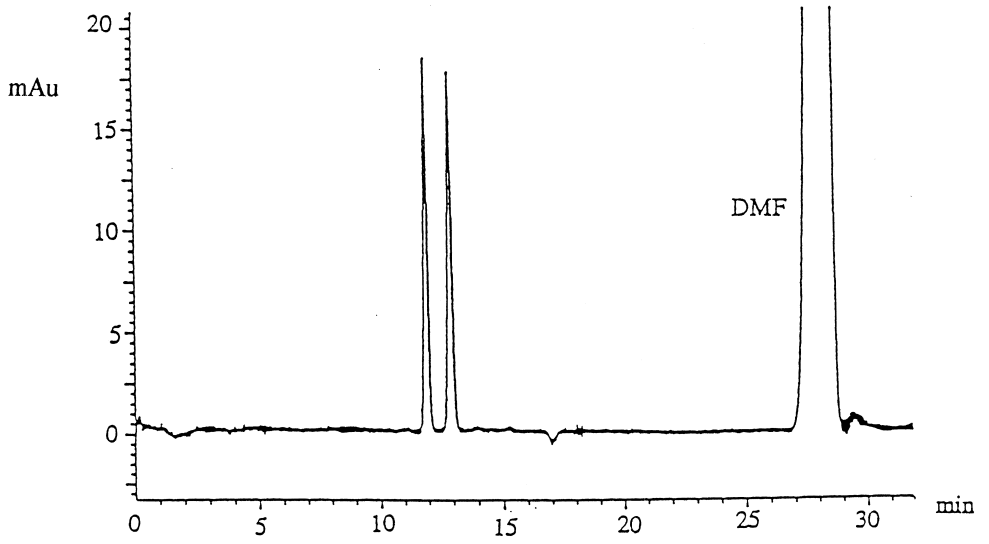

Fig. 3. Enantioseparation of naproxen Buffer: 34 m*M* phosphate buffer (pH 2.3) containing NH_2βCD (20 m*M*) and TMβCD (10 m*M*). Capillary: bare fused-silica, 38.5 cm (30 cm to the detector window) ×50 μm I.D.; temperature: 25°C, voltage: 20 kV; detection wavelength: 230 nm; hydrodynamic injection: 4 s. From Ref. [44].

taining CMβCD, the enantioseparation of aminoglutethimide was obtained (cf. Table 1). The neutral β-cyclodextrin provided the required chiral discrimination ability towards aminoglutethimide and the charged CMβCD provided the appropriate mobility. For Anigbogu et al., this kind of dual systems is similar to a MEKC system in the sense that resolution exhibits an optimum that is influenced by the size of the migration window.

The authors compared this dual CD system to a CD-MEKC system containing the achiral surfactant SDS and βCD [28] for the enantioseparation of aminoglutethimide and they found that the dual CD system presented several advantages. One of them is the ability to add a larger amount of methanol (MEKC can only tolerate 25% of organic solvent before micellization is disrupted). Another one is that analyte-CD interactions are generally more selective than solute– micelle interactions.

They also came to the conclusion that the buffer pH plays an important role in the enantioseparation of aminoglutethimide.

Nishi [16] investigated the separation of the enantiomers of three acidic binaphthyl compounds. Several CE modes such as cyclodextrin modified capillary zone electrophoresis (CD-CZE), micellar electrokinetic chromatography with CDs (CD-MEKC) and cyclodextrin mediated electrokinetic chromatography (CD-EKC) have been compared. The three racemic compounds were enantioseparated using a combination of αCD and TMβCD (10/10 m*M*) but also with a mixture of β-cyclodextrin phosphate (PO_4βCD) and αCD (10/10 m*M*). The highest resolution values were obtained using the three chiral selectors in combination in the BGE (PO_4βCD/αCD/TMβCD) (5/10/10 m*M*).

Gahm et al. [29] reported the successful use of mixtures of α-cyclodextrin (αCD) and sulfated β-cyclodextrins (SO_4βCD) as chiral selectors for the enantioseparation of monoterpenes (including α-pinene, β-pinene, camphene and limonene). The negatively charged β-cyclodextrin sulfate, which failed to resolve monoterpenes, acted as a non-specific carrier for these neutral solutes. The addition of α-CD to the buffer containing sulfated β-CD gave rise to high selectivity, resulting in remarkable chiral resolution (ranging from 4 to 25, cf. Table 1).

Fillet et al. studied the enantioseparation of a series of acidic drugs from different pharmacological classes, such as non-steroidal anti-inflammatory drugs (NSAIDs), barbiturates and anticoagulants [27,30–34].

These studies have shown that, by contrast with basic drugs, no or very poor enantiomeric resolution could be obtained for most acidic drugs when they were present in anionic form [27]. In a pH 3 buffer, these analytes were mainly present in uncharged form and therefore they were migrating with or very close to the EOF, the latter making their detection possible at the anodic side of the capillary (reversed polarity mode). Under these conditions, the use of a neutral CD (DMβCD or TMβCD) alone could not lead again to enantiomeric separation due to the lack of significant mobility difference between the free and the complexed forms of the analyte enantiomers.

However, the use of a negatively charged βCD derivative (SBβCD or CMβCD) in the pH 3 buffer was found to give rise to the formation of complexes migrating electrophoretically towards the anode and consequently this could lead to the enantiomeric resolution of these acidic compounds in uncharged form. However, it was found experimentally that the concentration of the anionic SBβCD or CMβCD had a limited influence on enantiomeric resolution [30,31]. A 5 m*M* concentration of SBβCD and a 10 m*M* concentration of CMβCD were found to give the analytes appropriate migration times (less than 10 min.) in a pH 3 phosphoric acid/triethanolamine buffer.

The addition of a neutral CD at a 10 m*M* concentration to this pH 3.0 buffer already containing SBβCD or CMβCD has been investigated. Two neutral CD derivatives (TMβCD and DMβCD) have proved to be particularly suited to the enantioseparation of acidic or neutral drugs [30] and have been preferably used.

Then, the concentration of the neutral cyclodextrin that gave the highest resolution was selected under the same conditions in the 1–50 m*M* range.

If the drug was neutral or had a very poor acidic character (e.g. barbiturates, with pKa values around 8), the use of a pH 5 buffer containing CMβCD (10 m*M*) and a neutral CD (TMβCD or DMβCD) was found to be sometimes more favourable for the separation selectivity [31].

However, all compounds examined, except chlormezanone, were completely enantioseparated using a dual CD system (combination of anionic and neutral CDs) in the pH 3 buffer. The most effective dual system at pH 3 for the acidic and neutral drugs examined was based on the simultaneous use of SBβCD and TMβCD, except for chlorthalidone (CMβCD/TMβCD), hexobarbital (SBβCD/DMβCD) and sulindac (SBβCD/DMβCD) (cf. Table 1).

The use of a pH 5 buffer was found to improve resolution values for all barbiturates and two NSAIDs (ibuprofen and indoprofen) and led to the complete enantioseparation of chlormezanone.

Fig. 4 illustrates the excellent separation of pentobarbital enantiomers by use of the dual system SBβCD/TMβCD (5/30 m*M*) at pH 3 or the system CMβCD/TMβCD (10/50 m*M*) at pH 5.

Surapanemi et al. [25] developed a theoretical model for the enantioseparation of neutral species based on the combined use of charged (SBβCD) and neutral (βCD, TMβCD and HPβCD) cyclodextrins. The model is based on simultaneous multiple equilibria involving the neutral analyte, the charged CD and the neutral CD. Selectivity and resolution equations were presented and it was demonstrated that the model can predict the migration times and the enantioresolution for the neutral analytes.

The enantioseparation of the chiral drugs tested (LY213829 and its sulfoxide metabolites) improved considerably in the presence of βCD, especially at low SBβCD concentration. At higher SBβCD concentrations, while keeping the concentration of βCD constant, the resolution decreased significantly.

Izumoto et al. [35] studied the enantioseparation of several drugs (basic as well as acidic compounds of various kinds) using neutral and charged CDs. Sulfated cyclodextrin (SO_4βCD) alone already gave interesting resolution for a wide variety of compounds, probably due to the relatively high degree of substitution (between 7 and 11 sulfate groups according to the manufacturer), which favours ionic interactions contributing to the enantiorecognition. The addition of a neutral CD to the acidic buffer further improved the enantioseparation but resulted in an increase in migration times.

The effect of the size of electrically neutral CDs (αCD, βCD, γCD and hydroxypropyl derivatives), as well as the influence of the substituents and the degree of substitution (hydroxypropyl-, dimethyl- and trimethyl-cyclodextrins) and the concentration of these different CDs were also studied [35].

Recently, Fillet et al. [19] described the separation

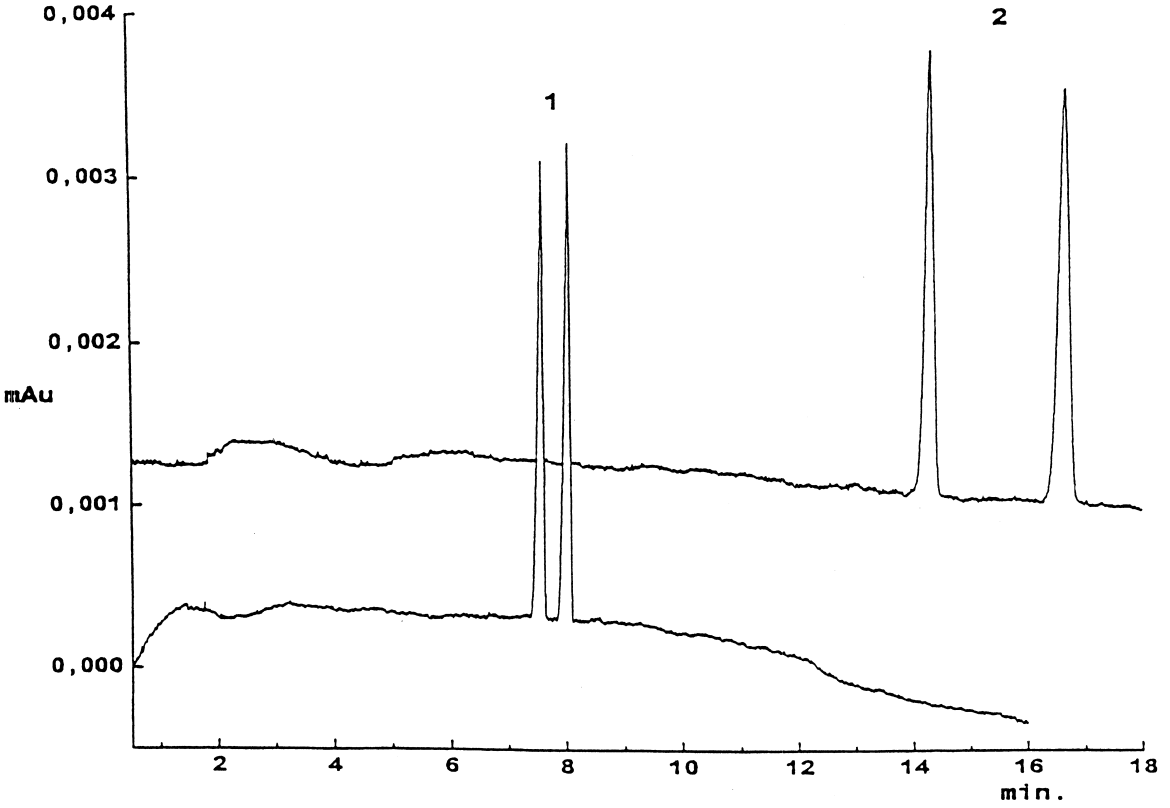

Fig. 4. Enantioseparation of pentobarbital Buffers: (1) 100 m*M* phosphoric acid/triethanolamine (pH 3.0) containing SBβCD (5 m*M*) and TMβCD (30 m*M*) (2) 100 m*M* phosphoric acid/triethanolamine (pH 5) containing CMβCD (10 m*M*) and TMβCD (50 m*M*). Capillary: bare fused-silica, 44 cm (37 cm to the detector window) ×50 μm I.D.; temperature: 25°C, voltage: −25 kV; detection wavelength: 210 nm; hydrodynamic injection: 5 s; Sample: $5 \cdot 10^{-5}$ *M* of racemic pentobarbital. From Ref. [31].

of a nonracemic mixture of a series of basic drugs (brompheniramine, chlorpheniramine, dimethindene, ephedrine and verapamil).

Dimethindene enantiomers were tested in the presence of CMβCD and of SBβCD. These two chiral selectors gave an opposite affinity pattern for the enantiomers. Their use in combination results in a decrease of separation selectivity (total loss of resolution) [19]. This due to the fact that both of these chiral selectors have the same effect on the mobility of the analyte (both decelerate it). On the contrary, two chiral selectors with the same mobility effect (and with the same recognition pattern) seem to be beneficial for this separation. Actually, an improvement in selectivity was observed with a combination of CMβCD and TMβCD.

The separation of a nonracemic mixture of chlorpheniramine enantiomers has also been reported using CMβCD as a carrier and alternatively, βCD or TMβCD as the second chiral selector [19]. In these dual systems, both chiral selectors decelerate the analyte. In the case of opposite chiral recognition pattern (CMβCD/TMβCD) the selectivity decreased and in the case of the same chiral recognition pattern (CMβCD/βCD) the selectivity was found to increase in such systems (cf. Table 1).

5. Conclusions

Dual cyclodextrin systems can provide very good selectivity for the enantioseparation of various kinds of drugs but they seem to be especially useful in the case of neutral or ionizable solutes in uncharged

form. In such instances, the charge CD should preferably have either no enantioselectivity or a chiral recognition pattern opposite to that of the neutral CD. Most applications described so far have been based on a trial and error approach. However, theoretical models have been developed which allow one to design dual cyclodextrin systems giving rise to highly selective enantioseparations.

Further studies involving other instrumental techniques (NMR spectroscopy, MS, X-ray crystallography, . . .) can also be useful for a better understanding of the inter molecular interactions involved in CE enantiomeric separations based on the use of mixtures of neutral and charged CDs.

6. Abbreviations

BDHP	1,1′-binaphthyl-2,2′-diyl hydrogenphosphate
CD:	cyclodextrin
CMβCD	carboxymethyl-β-cyclodextrin
DMβCD	dimethyl-β-cyclodextrin
HPαCD	hydroxypropyl-α-cyclodextrin
HPβCD	hydroxypropyl-β-cyclodextrin
HPγCD	hydroxypropyl-γ-cyclodextrin
HSβCD	heptakis-6-sulfato-β-cyclodextrin
NH_2 βCD	6-monoamino-6-deoxy-β-cyclodextrin
PO_4βCD	β-cyclodextrin phosphate
SBβCD	sulfobutyl-β-cyclodextrin
SO_4βCD	β-cyclodextrin sulfate
TMβCD	trimethyl-β-cyclodextrin

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