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Review

Application of charged single isomer derivatives of cyclodextrins in capillary electrophoresis for chiral analysis

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ABSTRACT

The review focuses on the role of ionic or ionisable single isomer derivatives (SIDs) of cyclodextrins on the separation of chiral analytes in capillary electrophoresis (CE), covering the period since the year 2000. The advantages of using pure compounds are discussed, as well as the ways to optimise the separations in the context of a rational approach to these techniques. Specific attention is paid to the modulation of the selector–analyte interaction. The advantage due to a detailed knowledge of equilibria occurring in solution during the CE run is underlined, particularly in the case of the presence of metal complexes, as occurs in chiral ligand exchange capillary electrophoresis (CLECE).

Contents

1. Introduction

This review concerns papers published in this decade, thus starting from 2000 until nowadays, and reporting investigations which use as selectors charged pure cyclodextrin derivatives. Though several detailed reviews appeared recently involving either all the chiral separations [1–4], or only those involving specifically cyclodextrins [5–10], we thought to give an useful tool to the scientific community by this further review. Also by relying on the availability of these reviews, we can skip over the characteristics of cyclodextrins as a general class of compounds, whose peculiar properties are well known: we can thus consider the specific subject of the present review and its novelty.

The novelty, and thus the usefulness, of the present review concerns the word "pure", what in literature is also known as "single isomer derivative" (SID). Obtaining a single pure isomer is a big problem in the context of cyclodextrins, owing to the presence of more glucopyranosinic rings absolutely equivalent, except in their relative position. When adding the appropriate reagent for the functionalisation of these compounds, it is unavoidable to get mixtures of more isomers, differing one another in the degree of substitution, namely the numbers of substituted units obtained. Further, depending on the relative position of the substituted glucopyranosinic rings, there is the possibility of regioisomerism too. In order to reduce the synthesis cost, firms that commercialise CD derivatives, generally yield the mixtures of derivatives, as obtained

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from the synthetic procedures, with no further separation step. They are characterised, besides the nature of the functionalising unit and the glucopyranosinic position/s substituted, only by the average number of glucopyranosinic units which are derivatised. Thus, if a laboratory needs to use a pure derivative, it is almost mandatory to carry out an on purpose synthesis.

Generally, people involved in the field of the separation science is not involved in the field of carbohydrate syntheses, which, besides, are among the most demanding. The result is that the most of separations, carried out worldwide, use mixtures of selectors.

However, it should be considered that any even slight modification in the structure of the selector may deeply influence the stability of the complexes that it will form with the different analytes in an unforeseeable way, at the present state of the theoretical knowledge of the molecular recognition phenomenon. Thus, each selector will form its own complexes with their own stabilities and it is not possible to theoretically neither predict, nor explain the results of the experiments carried out. It is precluded any possibility to go inside the process of selector–analyte interaction by investigations carried out with complementary techniques, like NMR or optical techniques, to remain in the field of spectroscopy. Furthermore, though the experimental conditions that are used in the synthetic procedure can assure the reproducibility in the average substitution degree, they cannot assure nothing about the relative proportion of the single regioisomers: thus any batch is different one from another in its detailed composition.

Thus, if we want both reproducibility and understanding of what is going on during the separation run, we are forced to use a single pure isomer (SID) as selector. These advantages justify, in our opinion, to dedicate a specific review to this group of investigations.

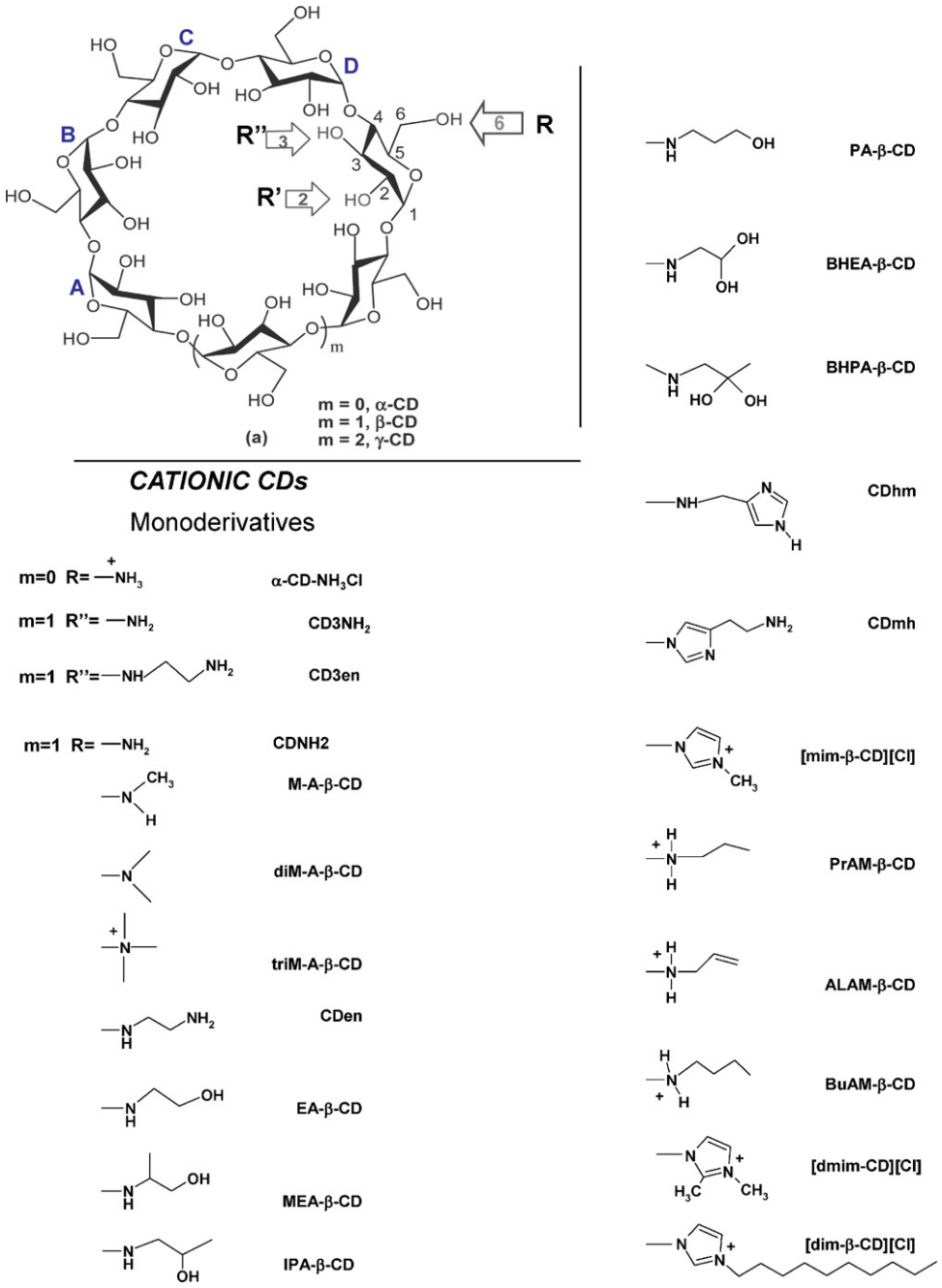

Fig. 1. Structures of cationic and anionic SID of cyclodextrins.

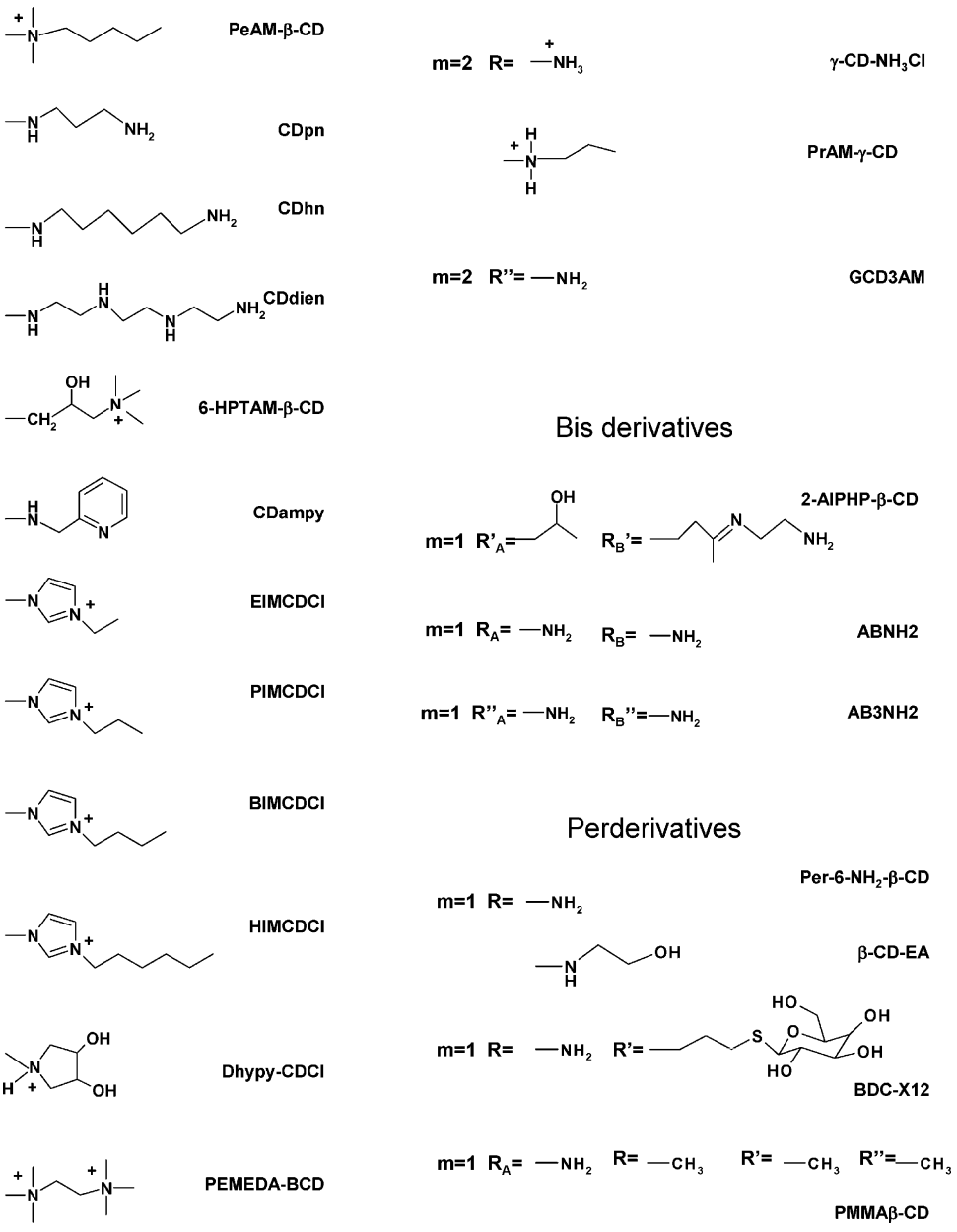

Fig. 1. (*Continued*)

The mechanism of selection by these kind of derivatives, owing to their electric charge, relies on the differential migration velocity between the free analyte and the analyte-selector complex. Thus, analogously to the separation carried out in the presence of charged micelles, it is possible to call this technique CD-EKC (cyclodextrin-modified electrokinetic chromatography).

The strategy involving the use of SID was systematically undertaken by the group of Vigh from 1997 [11], and developed in a series of papers described in the following of this review. This group choose to synthesise fully substituted derivatives (perderivatives) with the presence of sulphate groups, thus obtaining permanently polyanionic selectors. The advantage of this kind of selectors consists in assuring at any pH value a strong interaction with any cationic analyte, summing up to the analyte interaction of hydrophobic nature with the cyclodextrin cavity. Incidentally, it can be considered as a further advantage in the use of perderivatives, the easiness in their synthesis: by using a sufficient excess of functionalising reagent, all the glucopyranosinic rings will react. Generally, the elimination of the excess of functionalising reagent can be easily accomplished, often through a precipitation step. Any partial substitution, on the contrary, needs to carry out long and laborious steps of separations, often chromatographic, among the different products of the substitution reaction.

As will be seen in the pertinent section, these derivatives had a great success and were widely used, confirming the correctness of this strategy.

We would, however, without questioning this strategy, propose some observations that justify the use of alternative strategies and their greater synthetic difficulty.

We have just said that very strong interactions can occur between the selector and the analyte, but we should reflect if we are really interested in maximising their strength. By the theory of Wren and Rowe [12], since we are interested in the differential formation of the two diastereoisomeric complexes, we should find experimental conditions which maximise the difference in their formation degree rather than the formation degree of both of them. In this respect, a mono-derivative carrying one electric charge, can in some cases behave more selective than a polycharged perderiva-

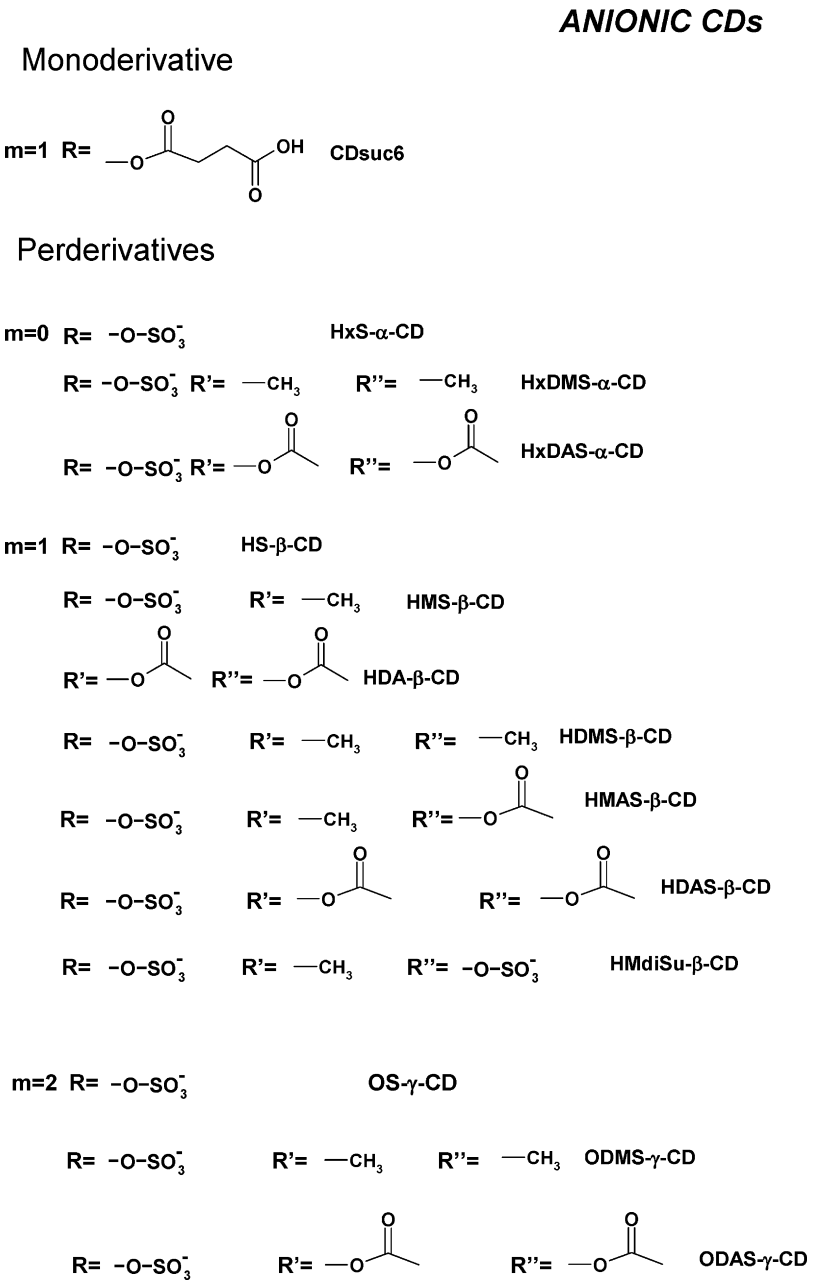

Fig. 1. (*Continued*).

tive selector. Furthermore, if the selector is permanently ionic, it is not mandatory to adjust the pH value of the BGE, at least as concerns the selector itself. This is particularly advantageous when the required state of protonation of the analytes dictates the pH value to be used.

Different strategies can be followed to modulate the strength of the analyte–selector interaction, like varying the analytical concentration of the selector and/or adding micelles to compete for interaction with the analytes. An additional way is possible, namely the modulation of the electrical charge of the same selector through the variation of the pH value of BGE. This is not possible with strong electrolytes since obviously in this case the pH value will not influence their state of protonation.

On the contrary, if, the weaker of the two interactions enantiomer–selector were too strong, it would be possible to decrease it, by slight pH changes in the range of the pK_A value of the selector, varying the concentration of the charged species, and thus its average fractional charge.

Obviously, accurate equilibria data in solution are required to exploit this kind of modulation, if possible concerning both the selector and the analytes. This approach was extensively applied by our group, on the basis of our expertise in the study of equilibria in solution by pH-potentiometry. This has permitted us to achieve what we call rational design of the separation experiments.

As can be easily argued, the knowledge of the equilibria occurring in the BGE during the electrophoretic run becomes much more important when several equilibria are occurring simultaneously. This is the typical case of the separation obtained by exploiting the ligand exchange mechanism, which will be considered in a specific section and where we address for more details.

The schematic molecular structures of the described SID are reported in Fig. 1.

2. CD-EKC

In 1989, for the first time, Terabe [13] used a charged derivative of β-CD to carry out the chiral separation of several enantiomeric pairs of dansyl amino acids (Dns-AAs), and early many other investigations followed [12,14–25]. Thus, from the early 90s, ionic or ionisable cyclodextrins have been increasingly used in CE. These compounds, not only show a dramatically increased aqueous solubility compared with that of parent CDs, but furthermore allow the separation of neutral enantiomers with no further additive component in BGE.

Applications of anionic and cationic CDs as chiral selectors in CE are summarized in Tables 1 and 2, respectively.

2.1. Anionic cyclodextrins

As a first step, Vigh et al. described the synthesis of three SID, namely, heptakis-6-*O*-sulpho-β-cyclodextrin (HS-β-CD) [26], heptakis(2,3-di-*O*-acetyl-6-*O*-sulpho)-β-cyclodextrin (HDAS-β-CD) [11] and heptakis(2,3-di-*O*-methyl-6-*O*-sulpho)-β-cyclodextrin (HDMS-β-CD) [27]. Afterwards, they described the synthesis of three SID of γ-cyclodextrin, namely octakis(2,3-di-*O*-acetyl-6-*O*-sulpho)-γ-cyclodextrin (ODAS-γ-CD) [28] octakis-6-*O*-sulpho-γ-cyclodextrin (OS-γ-CD) [29], and octakis (2,3-di-*O*-methyl-6-*O*-sulpho)-γ-cyclodextrin (ODMS-γ-CD) [30], as well as three SID of α-cyclodextrin, namely, hexakis(2,3-di-*O*-acetyl-6-*O*-sulpho)-α-cyclodextrin (HxDAS-α-CD) [31], hexakis(6-*O*-sulpho)-α-cyclodextrin (HxS-α-CD) [32] and hexakis(2,3-di-*O*-methyl-6-*O*sulpho)-α-cyclodextrin (HxDMS-α-CD) [33]. These selectors became commercially available and were successfully used for the separation of a large number of nonelectrolyte and weak electrolyte analytes in both low and high pH aqueous background electrolytes (BGEs) and acidified non-aqueous BGEs. Typical electropherograms for the chiral separations of some Dns-AAs in the presence of ODAS-γ-CD are shown in Fig. 2.

2.1.1. β-CD derivatives

The three 6-*O*-sulpho-β-CDs have been the most exploited derivatives of this cluster of nine 6-O-sulpho compounds, showing quite different behaviours towards the different analytes they separate. HDMS-β-CD showed good enantioselectivity in the separations of charged or highly polar chiral compounds, but it did not succeed in the separation of various naphthalene compounds [34]. HS-β-CD and HDAS-β-CD were successfully used for the enantioseparations of small peptides, also in comparison with other SID and randomly sulphated β-CD [35–37]. HDAS-β-CD, HS-β-CD and HDMS-β-CD were also used for electrophoretic chiral separation of pharmaceuticals [38–46] and of metabolites *in vivo*, *in vitro*, and in chemical synthesis [47], as well as in the enantioseparation of chiral intermediates [48] and in the analysis of the chiral purity of chemically different model compounds applied in homogeneous asymmetric hydrogenation catalysis [49]. Optimisation was achieved by modifying selected parameters, such as the buffer pH, concentration of the chiral selectors, type and amount of organic modifiers, type and concentrations of buffer, capillary size, temperature and applied voltage. Furthermore, NMR spectroscopy allows to give a deep insight in the understanding of the molecular recognition processes and thus to operate a fine tuning of the operational parameters. NMR and molecular modeling studies were performed to improve the level of understanding of the chiral recognition process occurring between linezolid and HDAS-β-CD [50–51]. NMR spectroscopy allowed not only to determine the stoichiometry and the binding constants of the complexes between HDAS-β-CD and *S*- or *R*-linezolid, but also, together with molecular dynamic simulations, to determine the mode of binding of linezolid to HDAS-β-CD and explaining the binding enantioselectivity (Fig. 3). The stereoselectivity is based on inclusion complexation where the main orientations of linezolid are those with oxazolidinone moiety immersed in HDAS-β-CD cavity. The opposite orientation is also possible but only inclusion through the oxazolidinone influences the enantioselectivity [51].

Table 1
Applications of anionic cyclodextrins in chiral CD-EKC.

Hexakis(6-*O*-sulpho)-α-CD	HxS-α-CD	Nonionic, weak-acid and weak-base analytes [32,33]
Hexakis(2,3-di-*O*-methyl-6-*O*-sulpho)-α-CD	HxDMS-α-CD	Nonionic, weak-acid and weak-base analytes [33]
Hexakis(2,3-di-*O*-acetyl-6-*O*-sulpho)-α-CD	HxDAS-α-CD	Nonionic, weak-acid and weak-base analytes [31–33,84]
6-O-succinil-β-CD	CDsuc6	Catecholamines [61]
Heptakis-(2,3-di-*O*-acetyl)-β-CD	HDA-β-CD	Peptides [36], deprenyl and selegiline [45]
Heptakis-6-*O*-sulpho-β-CD	HS-β-CD	Dipeptide and tripeptide [35–37], aryl alkyl and aryl benzyl sulphoxides [48], propafenone, diprafenone and analogues [57], antihistamines, antimalarias, β-agonists, β-blockers [58], profens [62,63], M3 antagonist [80–82], pharmaceutic compounds [40,41], atropine enantiomers [54], thienylpyrazolylethanamine derivatives [44], weak basic analytes [76], flavanones and flavanone-7-*O*-glycosides [56], catecholamines [59,60], atomoxetines [43], quinuclidine [46], omeprazole [53], model compounds applied in homogeneous asymmetric hydrogenation catalysis [49], phenothiazines [65]
Heptakis(2-*O*-methyl-6-*O*-sulpho)-β-CD	HMS-β-CD	Weak basic analytes [76]
Heptakis-(2,3-dimethyl-6-*O*-sulpho)-β-CD	HDMS-β-CD	Substituted naphthalene compounds [34], pharmaceutic compounds [38–41,67], propafenone, diprafenone and analogues [57], M3 antagonist [81,82], catechins and naphthols [64], β-blockers, local anesthetics, sympathomimetics [68,69], weak basic analytes [76], aziridines [66], omeprazole and 5-hydroxyomeprazole [52], quinuclidine [46], atropine, scopolamine, ipratropium and homatropine [55], mebeverine and salbutamol [71], timolol [70]
Heptakis(2-*O*-methyl-3-*O*-acetyl-6-*O*-sulpho)-β-CD	HMAS-β-CD	Weak-base pharmaceuticals [75], weak basic analytes [76]
Heptakis(2,3-diacetyl-6-*O*-sulpho)-β-CD	HDAS-β-CD	Dipeptide and tripeptide [35–37], pharmaceutic compounds [38], aryl alkyl and aryl benzyl sulphoxides [48], propafenone, diprafenone and analogues [57], pharmaceutic compounds [39–41], M3 antagonist [80–82], labetalol [78], nonionic, weak-acid and weak-base analytes [42,76,84], 6-oxycodol and nor-6-oxycodol [47], aziridines [66], selegiline [45], atropine, scopolamine, ipratropium and homatropine [55], mebeverine and salbutamol [71], linezolides [50,51]
Heptakis(2-*O*-methyl-3,6-di-*O*-sulpho)-β-CD,	HMdiSu-β-CD	Pharmaceutical weak-base enantiomers [72], cationic chiral analytes [73], Dns-Trp [74]
Octakis-6-*O*-sulpho-γ-CD	OS-γ-CD	Nonelectrolyte, acidic, basic, and ampholytic analytes [29,79], RR-M3 and SS-M3 [80,82], model compounds applied in homogeneous asymmetric hydrogenation catalysis [49]
Octakis(2,3-di-*O*-methyl-6-*O*-sulpho)-γ-CD	ODMS-γ-CD	Nonionic, weak-acid and weak-base analytes [30,83]
Octakis(2,3-diacetyl-6-sulpho)-γ-CD	ODAS-γ-CD	Neutral, acidic, basic, and amphoteric enantiomers [28,79], weak-base pharmaceuticals [77], pharmaceutic compounds [40], RR-M3 and SS-M3 [80,82], labetalol [78]

Table 2
Applications of cationic cyclodextrins in chiral CD-EKC and in CLECE.

6-Mono-deoxy-6-ammonium-α-CD chloride	α-CD-NH_3Cl	Dns-AAs, propionic acids [96], Dns-AAs, anionic and ampholytic analytes [104]
6-Mono-deoxy-6-amino-β-CD	CD-NH2	Anionic and ampholytic analytes [99,102], Dns-AAs, anionic and ampholytic analytes [104]
3A-Mono-deoxy-3A-amino-2A(S),3A(R)-β-CD	CD3NH2	AAs [115,129]
6-Mono-deoxy-6-monomethylammino-β-CD	M-A-β-CD	DNP-AAs [116]
6-Mono-deoxy-6-dimethylammino-β-CD	diM-A-β-CD	DNP-AAs [116]
6-Mono-deoxy-6-trimethylammino-β-CD	triM-A-β-CD	DNP-AAs [116]
6-Mono-deoxy-(2-aminoethylamino)-β-CD	CDen	Phenoxy acids [120], AAs [115], AAs by CLECE [131,136], Dns-AAs [124], Dns-AAs by CLECE [137]
3-Mono-deoxy-3-[1-(2-amino)ethylamino]-2A(S),3A(R)-β-CD	CD3en	AAs [131]
6-Mono-deoxy-6-(2-hydroxy)ethylamino-β-CD	EA-β-CD	Mandelic acid, pyrethroic acids [108], profens [109]
6-Mono-deoxy-6-(1-methyl-2-hydroxy)ethylamino-β-CD	MEA-β-CD	Mandelic acid, pyrethroic acids [108]
6-Mono-deoxy-6-(propylammonium)-β-CD	PrAM-β-CD	Hydroxy and carboxylic acids, amphoteric analytes [96,103]
6-Mono-deoxy-6-(2-hydroxy)propylamino-β-CD	IPA-β-CD	Mandelic acid, pyrethroic acids [108], profens [109,111,114]
6-Mono-deoxy-6-(3-hydroxy)propylamino-β-CD	PA-β-CD	Mandelic acid, pyrethroic acids [108], profens [109–111,114]
6-Mono-deoxy-6-[bis(2-hydroxy)ethyl]amino-β-CD	BHEA-β-CD	Mandelic acid, pyrethroic acids [108]
6-Mono-deoxy-6-[bis(2-hydroxy)propyl]amino-β-CD	BHPA-β-CD	Mandelic acid, pyrethroic acids [108]
6-Mono-deoxy-6-N-allylammonium-β-CD	ALAM-β-CD	AAs and Dns-AAs [98]
6-Mono-deoxy-6-[4-(2-aminoethyl)imidazolyl]-β-CD	β-CDmh	AAs [128,131]
6-Mono-deoxy-6-[2-(4-imidazolyl)ethylamino]-β-CD	β-CDhm	AAs [131]
6-Mono-deoxy-butylammonium-β-CD tosylate	BuAM-β-CD	α-Hydroxy acids, carboxylic acids and ampholytic analytes [100]
6-Mono-deoxy-6-(3-methyl-imidazolium)-β-CD chloride	[mim-β-CD][Cl]	Dns-AAs [91–94]
6-Mono-deoxy-6-(1,2-dimethyl-imidazolium)-β-CD chloride	[dmim-CD][Cl]	Dns-AAs [91]
6-Mono-deoxy-(3-ethylimidazolium)-β-CD	EIMCDCl	Dns-AAs [93]
6-Mono-deoxy-(3-propylimidazolium)-β-CD	PIMCDCl	Dns-AAs [93]
6-Mono-deoxy-(3-butylimidazolium)-β-CD	BIMCDCl	Dns-AAs [91,95]
6-Mono-deoxy-(3-hexylimidazolium)-β-CD	HIMCDCl	Dns-AAs [93]
6-Mono-deoxy-6-(3-decyl-imidazolium)-β-CD chloride	[dim-β-CD][Cl]	Dns-AAs [91]
6-Mono-deoxy-6-*N*,*N*,*N*′,*N*′,*N*′-pentamethylethylenediammonio-β-CD	PEMEDA-BCD	Anionic, weak-acids and neutral analytes [117]
6-Mono-deoxy-N-pentylammonium-β-CD chloride	PeAM-β-CD	Anionic and ampholytic analytes [101]
6-Mono-deoxy-6-[1-(3-amino)propylamino]-β-CD	CDpn	Dns-AAs by CLECE [137]
6-Mono-deoxy-6-[1-(6-amino)hexylamino]-β-CD	CDhn	Dns-AAs by CLECE [137]
6-Momodeoxy-6-[1-(2,4-diamino)diethyldiamino]-β-CD	CDdien	Dns-AAs by CLECE [137]
6-O-(2-hydroxy-3-trimethylammoniopropyl)-β-CD	6-HPTMA-β-CD	Acidic compounds [113]
6-Mono-deoxy-6-[N-(2-methylamino)pyridine]-β-CD	CDampy	AAs [115,131]
2-O-(2-Aminoethyl-imino-propyl)-β-O-hydroxypropyl-β-CD	2-AIPHP-β-CD	Profens and acidic compounds [112]
6-Mono-deoxy-6-(3*R*,4*R*-dihydroxypyrrolidine)-β-CD	Dhypy-CDCl	Dns-AAs, anionic and ampholytic acids [105]
6A,6B-Dideoxy-6A,6B-diamino-β-CD	ABNH2	AAs [131]
3A,3B-Dideoxy-3A,3B-diamino-2A(S),2B(S),3A(R),3B(R)-β-CD	AB3NH2	AAs [130,131]
6A,6D-(6,6′-Diamino-α,α′-trehalose)-6A,6D-dideoxy-β-CD	THAMH	Phenoxy acids [120], profen [122]
6A,6D-Dideoxy-6A,6D-[6,6′-dideoxy-6,6′-di(*S*-cysteamine)-α,α′-trehalose]-β-CD	THCMH	Phenoxy acids [120,121], profens [122], Dns-AAs [124]
6A,6D–Dideoxy-6A,6D-N-[6,6′-di-(β-alanylamido)-6,6′-dideoxy-α,α′-trehalose]-β-CD	THALAH	Phenoxy acids [120], profens [120], Dns-AAs [123]
Heptakis(6-deoxy-6-amino)-β-CD	per-6-NH_2-β-CD	Carboxylic acids, profens, Dns-AAs [89]
Heptakis(6-deoxy-6-hydroxyethylamino)-β-CD	β-CD-EA	Carboxylic acids and anionic analytes [88]
Heptakis(6-deoxy-6-amino-2-galactosyl)-β-CD	BDC-X12	Nucleotide [90]
Permethylmonoamino-β-CD	PMMAβ-CD	Pyrethroic acids [118], profens [63]
6-Mono-deoxy-6-N-ammonium-γ-CD chloride	γ-CD-NH_3Cl	Dns-AAs, propionic acids [96], Dns-AA, anionic and ampholytic analytes [104]
3-Mono-deoxy-3-amino-2(S),3(R)-γ-CD	GCD3AM	Dns-AAs [106,107]
6-Mono-deoxy-6-propylammonium-γ-CD chloride	PrAM-γ-CD	Dns-AAs, propionic acids [96]

Enantiomeric determination of omeprazole and its metabolite 5-hydroxyomeprazole was achieved by HDMS-β-CD in acidic methanol [52] and by HS-β-CD in aqueous BGE with MS detection [53]. The aqueous CE method was developed and designed to afford increased compatibility with ESI–MS detection, employing an ammonium acetate buffer system (pH 5.8).

Among pharmaceuticals, atropine enantiomers were resolved by using comparatively randomly sulphated CDs and HS-β-CD [54]. Enantioseparations of atropine, homatropine, scopolamine and ipratropium were optimised by using a standard oil-in-water (*O*/*W*) microemulsion (MEEKC) and varying the nature and concentration of HDMS-β-CD or HDAS-β-CD as well as the organic modifier (methanol, 2-propanol or ACN) [55]. High resolution and short migration times of all tropa alkaloids were achieved by using HDMS-β-CD and SCD in the microemulsion BGE and were superior to corresponding CD-modified CE methods. Analogously, flavanones and flavanone-7-*O*-glycosides were successfully separated by micellar electrokinetic chromatography (MEKC) with sodium cholate as chiral surfactant [56], in the presence of a series of CD derivatives, including SID HS-β-CD.

Sometimes, randomly substituted highly sulphated cyclodextrins succeed in the enantiomeric separation, whereas SID do not. For example, antiarrhytmic drugs propafenone (PF), diprafenone (DF) and their metabolites were better enantioseparated by randomly sulphated β-CD than by HDAS-β-CD, HDMS-β-CD and HS-β-CD [57], whereas doxylamine was resolved by HS-β-CD only. On the contrary, alprenolol was resolved by the randomly sulphated CD, but not by HS-β-CD [58]. Analogously, DL-norepinephrine, DL-epinephrine, and DL-isoproterenol and three structurally related compounds (DL-octopamine, DL-synephrine, and DL-norephedrine) were better separated by randomly sulphated CD than by HS-β-CD [59–60], suggesting that sulfate substituents of SCD located at the C2-position (and perhaps C3-position), which interact strongly with the diol moiety of cat-

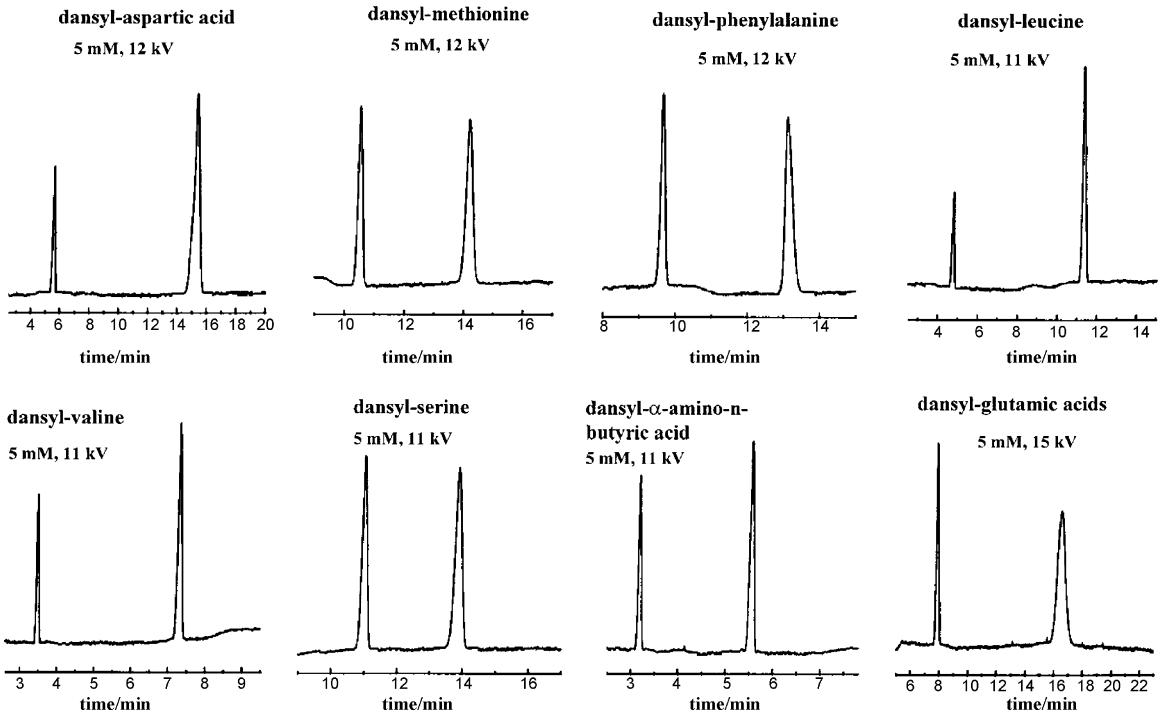

Fig. 2. Typical electropherograms of chiral amphoteric analytes. The numbers next to the structures indicate the ODAS-γCD concentrations (mM) and the applied effective potentials (kV). All measurements were carried out in the 25 mM phosphoric acid buffer the pH of which was adjusted to 2.5 with LiOH. Capillary: 25 μm i.d., 19 cm/26 cm effective/total length, uncoated fused silica.
Reported with permission from Ref. [28].

echolamines, may play an important role in enantioseparation and chiral recognition of these analytes. Catecholamines were also separated by 6-*O*-succinil-β-cyclodextrin (CDsuc6) [61]. The CE experiments at pH 5.6 showed very promising selector ability by CDsuc6 for the chiral recognition of all the catecholamines tested, while at pH 9.2, only racemic terbutaline was successfully separated. It is readily apparent that at this pH, formation degrees are much lower than in acidic pH. The reason for this lowering of stability can likely be ascribed to the fact that at pH 9.2 the catecholamines are present almost exclusively as neutral species. Only terbutaline, probably owing to its bulky alkyl moiety, gives rise to a complex with the selector, rather stable to obtain the separation (Fig. 4).

The enantioseparation of a series of NSAIDs (non-steroidal anti-inflammatory derivatives), namely fenoprofen, flurbiprofen, ibuprofen, and ketoprofen [62–63], was obtained by employing a dual cyclodextrin system by using the HS-β-CD together with either the neutral CD derivative, heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD) [62], or with the SID cationic permethyl-6-monoamino-6-mono-deoxy-β-CD (PMMA-β-CD) [63]. Catechins and naphtols were also resolved by using a mixture of neutral and anionic β-cyclodextrins [64] and effective enantioseparation of phenothiazines can be advantageously and favourably achieved by coupling HS-β-CD with some neutral CDs [65]. Comparative studies with single CD and dual CD systems containing a neutral CD and HS-β-CD or randomly sulfate-substituted β-CD, clearly showed that enantioseparations of phenothiazines, except for methotrimeprazine, could be effectively and more favourably achieved with the dual CD systems containing the SID than with the dual CD systems containing the SCD [65].

The problem of enantioseparation of sparingly soluble drugs was addressed by NACE (non-aqueous capillary electrophoresis)

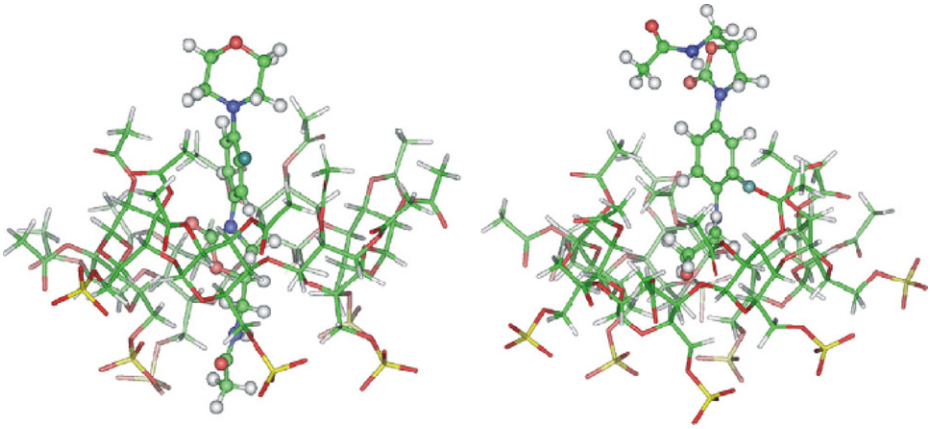

Fig. 3. The closest to the average snapshots geometries from 40 ns molecular dynamic calculation for linezolid/HDAS-β-CD complexes.
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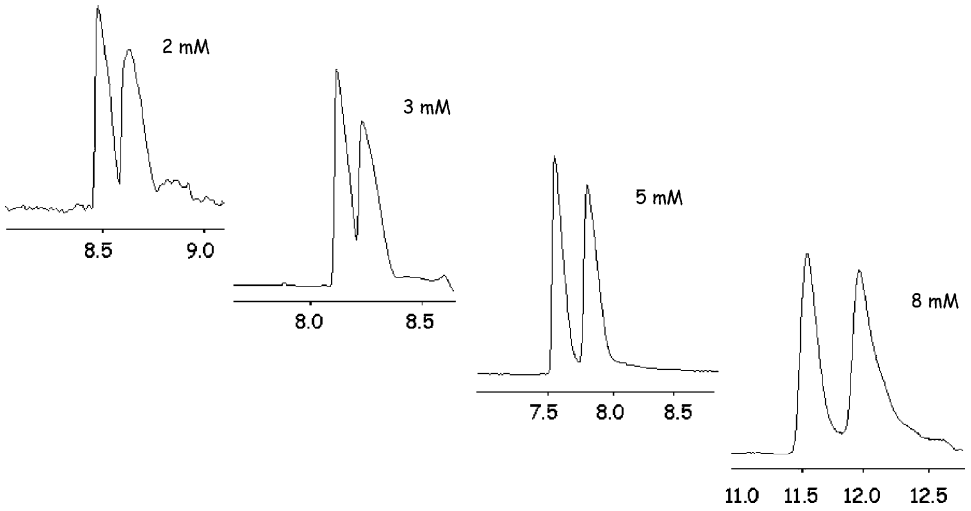

Fig. 4. Electropherograms of terbutaline racemate in the presence of CDsuc6 at pH 9.2 BGEs for the chiral separation experiments were prepared by dissolving CDSuc6 (2.0–8.0 mM) in 10.0 mM borate buffer (pH 9.2). Capillary: 75 μm i.d., 49 cm/60 cm effective/total length, uncoated fused silica
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technique. Since the solubility of all aziridines is limited in aqueous phosphate buffer, a robust method was set up using HDAS-β-CD in acidic methanol, obtaining very good resolution in a rather short analysis time [66]. The coupling of camphor-sulphonate$^-$ and the anionic SID HDMS-β-CD resulted in a powerful tool to achieve the enantioresolution of β-blockers (such as atenolol, celiprolol, propranolol and timolol) and local anesthetics (such as bupivacaine, mepivacaine and prilocaine) as well as mebeverine in NACE [67–71].

Another member of SID is heptakis-(2-*O*-methyl-3,6-di-*O*-sulpho)-β-CD (HMdiSu-β-CD) [72–74]. HMdiSu-β-CD is a highly charged CD with 14 bulky sulphate substituents on both the primary and secondary CD rims. The bulky substituents on both sides of the cavity entrance may hinder inclusion complex formation between chiral analytes and HMdiSu-β-CD. Generally, HMdiSu-β-CD does not seem to be a powerful chiral selector as other randomly and selectively sulphated CD derivatives. The lower ability of HMdiSu-β-CD as a chiral selector compared to other members of the family of SID [26–27] can be explained by the lower hydrophobicity of its cavity due to the high number of directly bonded sulphate groups as well as due to the steric hindrance on both its rims. Both of these effects may disfavour the formation of an inclusion complex in aqueous solution. In addition, the substituents located in positions 2 and 3 of the glucose units may disturb hydrogen bond formations between the neighbouring glucose units. This may lead to less structural rigidity of the CD cavity. In NMR experiments on these systems, no complexation-induced splitting of the resonance signals was observed for the protons of the cationic chiral analytes investigated, but a moderate enantioseparation of the analytes was observed in CE, thus suggesting that the CD cavity is not involved in the complex formation [73].

SID sulfated β-cyclodextrins carrying non-identical substituents at all of the C2, C3, and C6 positions, namely heptakis(2-*O*-methyl-3-*O*-acetyl-6-*O*-sulpho)-β-cyclodextrin (HMAS-β-CD) [75] and heptakis(2-*O*-methyl-6-*O*-sulpho)-β-cyclodextrin (HMS-β-CD) [76] have been synthesised and used for the capillary electrophoretic separation of the enantiomers of a group of 24 weak basic pharmaceuticals in acidic aqueous and acidic methanolic background electrolytes. HMAS-β-CD interacted more strongly than HDMS-β-CD with most of the analytes studied, but less strongly than HDAS-β-CD, the analogous SID with identical substituents at the C2 and C3 positions. On the other hand, HMS-β-CD interacted strongly with only about half of the analytes studied and did not prove to be as good a resolving agent in acidic methanolic BGEs as HMAS-β-CD.

2.1.2. γ-CD derivatives

To provide SID with a larger cavity, sulphated γ-cyclodextrins (ODAS-γ-CD, OS-γ-CD, ODMS-γ-CD) were synthesised and used for CD-EKC enantiomer separations.

The sodium salt of octakis-(2,3-di-*O*-acetyl-6-*O*-sulpho)-γ-cyclodextrin (ODAS-γ-CD) [28] and the sodium salt of octakis-6-*O*-sulpho-γ-cyclodextrin (OS-γ-CD) [29] have been synthesised and used to separate the enantiomers of a variety of noncharged, weak-acid, weak-base, and amphiprotic analytes in low pH BGEs. It is noteworthy that ODAS-γ-CD afforded much greater peak resolution values for the Dns-AAs than the corresponding HDAS-β-CD [28] and fast separations with good peak resolution values for weak-base analytes in NACE [77]. Furthermore, it has been demonstrated that using the SID ODAS-γ-CD in the running buffer a complete separation of all four stereoisomers of labetalol was achieved: ODAS-γ-CD afforded fast separations, with good peak resolutions and in a shorter amount of time than that has been previously achieved with other chiral additives [78]. Hence, the bigger cavity of ODAS-γ-CD resulted in a better separation of the four stereoisomers of labetalol.

OS-γ-CD interacts with many analytes differently than its counterpart, ODAS-γ-CD, and its analogous derivative of β-CD, HS-β-CD. Often, selectivity values observed with OS-γ-CD were different from those of other SID, like ODAS-γ-CD, HS-β-CD or HDAS-β-CD. Adequate, fast separations were obtained with OS-γ-CD in the high pH background electrolyte for a large number of analytes [79].

The different binding characteristics of ODAS-γ-CD and OS-γ-CD were well elucidated by Zhou et al by a combined study using CE, proton, ^{19}F and ^{13}C NMR, and infrared (IR) spectroscopy [80] on the chiral recognition of 2-(*R*)-*N*-[1-(6-aminopyridin-2-ylmethyl)piperidin-4-yl]-2-[(1*R*)-3,3-difluorocyclopentyl]-2-hydroxy-2-phenylacetamide (RR-M3), and its enantiomer (SS-M3), previously studied with other single isomer β- and γ-derivatives [81–82]. The overall NMR (^{1}H, ^{19}F and ^{13}C) data suggest that neither RR-M3 nor SS-M3 are fully included inside the ODAS-γ-CD cavity, the main hydrophobic interaction occuring between the acetyl moiety on ODAS-γ-CD and the difluorocyclopentyl ring of the guest. However, the phenyl

moiety of the RR-M3 or SS-M3 is deeply included into the OS-γ-CD cavity with a sufficient hydrophobic interaction between difluorocyclopentyl ring of the guest and OS-γ-CD cavity. Ion pairing interactions occur between the positively charged amino site of the guest and the negatively charged sulphonate site of the host. These results showed sufficient differences between the structures of the M3 complexes with ODAS-γ-CD and OS-γ-CD. These differences may be responsible for the opposite affinity of RR-M3 and SS-M3 towards these two CDs. The CE results, in fact, indicated that the reversal of migration order is not simply due to a change of CE parameters. NMR and IR data highlighted a 1:1 complex stoichiometric ratio of RR-M3 and SS-M3 for both CDs and that differences in the structure of the complexes are formed may be responsible for the opposite migration order for the enantioseparation of RR-M3 and SS-M3.

The separation selectivities obtained with ODMS-γ-CD were different from those obtained with either ODAS-γ-CD or OS-γ-CD or HDMS-β-CD, indicating that all of these SID sulfated CDs have specific applications in the CE separations of enantiomers [30]. Though ODMS-γ-CD interacts less strongly with many of the analytes tested than the other members of SID of γ-cyclodextrin, like OS-γ-CD and ODAS-γ-CD, it permits to obtain excellent selectivities, often complementary to those of both the β-cyclodextrin and γ-cyclodextrin SID. Rapid, efficient enantiomer separations were observed for a large number of structurally different analytes in acidic aqueous background electrolytes [30].

However, ODMS-γ-CD appears to interact with most of the analytes less strongly with respect of other described selectors, structurally similar, especially in high pH BGE [83].

2.1.3. α-CD derivatives

In order to further investigate the role of the cavity size in the enantiomeric separation, sulphated α-cyclodextrins (HxDAS-α-CD, HxS-α-CD, HxDMS-α-CD) were synthesised and used in CD-EKC.

HxDAS-α-CD, HxS-α-CD and HxDMS-α-CD interact less strongly with many of the analytes tested than the analogous β- and γ-cyclodextrin derivatives, namely, HDAS-β-CD, HS-β-CD and HDMS-β-CD and ODAS-γ-CD, OS-γ-CD and ODMS-γ-CD. Nevertheless, they succeeded in the separation of the enantiomers of a large number of weak electrolyte and nonelectrolyte analytes in acidic aqueous background electrolytes [31–33]. For some of the analytes, the separation selectivities obtained with HxS-α-CD were complementary to those observed with HxDAS-α-CD, HS-β-CD, and OS-γ-CD [32]. For all the analytes, the effective mobilities and separation selectivities as a function of the background electrolyte concentration of HxDAS-α-CD followed the trend that was found for HDAS-β-CD and ODAS-γ-CD. HxDAS-α-CD was also used in NACE [84], showing by the selectivity values found for some of the weak basic analytes tested, that the acidic aqueous and acidic methanol BGEs are each other complementary in their separation abilities.

2.2. Cationic Cyclodextrins

Cationic CDs as chiral selectors have been much less used compared to anionic CDs, as can be seen by the pertinent references in Table 2. The first examples of cationic SID appearing in literature for CD-EKC applications were amino-functionalised β-CDs, such as the 6A,D-dimethylamino-β-CD [85], 6-amino-β-CD [86] and 6-deoxy-6-*N*-histamino-β-CD and 6-deoxy[4-(2-aminoethyl)imidazolyl]-6-*N*-histamino-β-CD [87].

Cationic CDs are either strong-electrolyte, like those functionalised with quaternary ammonium groups, or weak electrolytes. Just as other CD derivatives, cationic CDs can be randomly substituted or SID.

2.2.1. Open-chain derivatives

SID polycationic-β-CDs, completely aminated in position six of the glucopyranosinic unit, were first investigated in CD-EKC because these symmetric compounds show good batch-to-batch reproducibility and good enantioseparation abilities [88–90]. However, from an enantiorecognition point of view, the mono-functionalised SID cationic CDs have the advantage that the electrostatic interactions are localised to a well-defined part of the CD. In mono-functionalised CDs, charge localisation creates a non-symmetrical chemical environment, which provides greater stereodifferentiation in the intermolecular interactions between the analyte and the selector than in the persubstituted CDs.

The research group of Siu-Choon Ng has been actively engaged in developing selectively modified monosubstituted α, β and γCDs as chiral selectors for chiral chromatographic and electromigration techniques. A wide range of single isomer monosubstituted positively charged CDs have been explored by introducing imidazolium [91–97], ammonium [98–104] moieties with varied alkyl chain length, as well as a dihydroxypyrrolidine substituent [105]. This class of compounds has been widely used to separate α-hydroxy acids, carboxylic acids and ampholytic analytes, as well as amino acids and Dns-AAs, both as single racemate, and as mixtures, at different pH values (Fig. 5). Chiral separation of Dns-AAs was found to be highly dependent on pH, since the degree of protonation of these analytes can alter the strength of electrostatic interaction and inclusion complexation between each enantiomer and chiral selector. The chiral resolution of α-hydroxy acids decreased with increasing the BGE pH, whereas Dns-AAs displayed a maximum resolution at pH 6.0 [100], indicating the existence of secondary interactions (*e.g.*, hydrogen bonding) between CD and analytes, which are affected by the acidity of BGE. Furthermore, the binding constants of the complexes formed by ammonium-β-CDs with these anionic and ampholytic analytes, calculated by applying the *x*-reciprocal method [99], suited well with the mobility difference model [12], concerning the existence of a maximum in the selectivity at a specific concentration of chiral selector.

In CD chemistry, the most critical factors influencing the stability of the inclusion complex are the size, shape, rigidity, polarity and steric hindrance of the guest analytes, as well as of the cyclodextrin host molecules. Hence, the dimension of the cavity may have significant effects on the separation ability of CD-based chiral selectors and thus, in order to study the influence of the cavity size on the resolution abilities of CD derivatives towards model analytes, in addition to β, several α and γ cationic cyclodextrins have been synthesised and exploited in CE [96,104,106,107]. Most dansyl amino

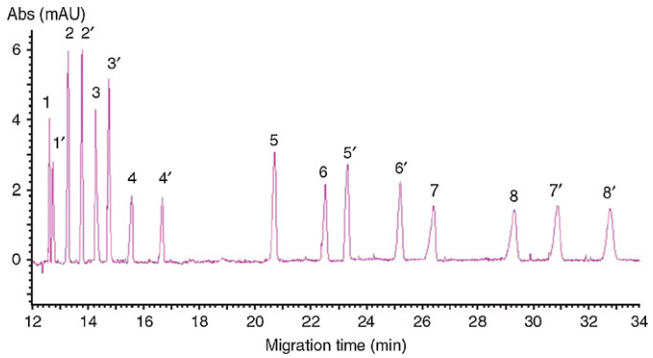

Fig. 5. Baseline separation of an eight-acid mixture using β-CD-NH_3Cl with PDA detection at 254 nm. Legends: 1,1′: dansyl-DL-α-aminocaprylic acid cyclohexylammonium salt; 2,2′: dansyl-DL-α-amino-n-butyric acid piperidinium salt; 3,3′: 2-naphthylmethoxyacetic acid; 4,4′: DL-3-phenyllactic acid; 5,5′: dansyl-DL-glutamic acid bis(cyclohexylammonium) salt; 6,6′: 3-hydroxy-4-methoxymandelic acid; 7,7′: DL-mandelic acid; 8,8′: 4-hydroxy-3-methoxymandelic acid. Conditions: 20 mM CD, 50 mM phosphate buffer (pH 6.0).
Reported with permission from Ref. [104].

acids were resolved to some extent when γ- and β-CD derivatives were used as chiral selectors. Conversely, α-CD derivatives can only give very poor resolution for all the analytes. More interestingly, better resolutions were generally obtained with a γ-CD chiral selector when compared with its β-CD analogue, due to the 'tight-fit' inclusion. In particular, 3-deoxy-3-amino-2(S),3(R)-γ-cyclodextrin (GCD3AM) shows very good chiral recognition ability even at very low concentrations and on wide range of pH values. Chiral baseline resolution for some Dns-AAs was obtained even at a selector concentration as low as 0.05 mM, lower than the sum of the concentrations of the enantiomers, while values of resolution as high as 18.68 were obtained (Fig. 6) [107]. The higher values of selectivity observed at neutral pH, where opposite charges are present between host and guest, clearly show the important role played by the electrostatic interaction, which, though it is not the only selector–analyte interaction occurring, plays a role of assistance and reinforcement of the complex, acting as a steric constraint and improving the chiral selectivity.

Application of CDs as chiral selectors in the enantiorecognition process is fundamentally based on complexation (by inclusion or external) of a part of the analyte and various interactions between analyte and functional moieties (hydroxyls or different substituents) of the CD rims. Therefore, position and structure of only one substituent can play an important role in the enantioseparation ability of a CD derivative. In the monoderivatised cationic β-CD derivatives containing amino function, the amino group usually occurred directly and/or by a spacer linked to the primary rim of the β-CD. Thus, the positive charge directly linked to the CD rim give an enantioselective electrostatic interaction with the anionic part of the analyte. Iványi et al. synthesised a family of SID 6-mono-N-(hydroxyalkyl)-amino-β-CDs [108–111], studying the effect of steric hindrance and hydrogen bond formation on enantioseparation, and thus permitting the choice of the appropriate chiral selectors for each racemate. All the compounds showed higher, but different enantioselectivity depending on the number and size of substituents bound to the amino N-atom. The presence of one hydroxyalkyl group significantly increased both the enantioselectivity and the resolution compared to the primary amino-β-CD, while two hydroxyalkyl moieties decreased them due to the predominance of steric hindrance. Also in this case, the problem of enantioseparation of sparingly soluble drugs, such as profen, was addressed by the development of non-aqueous BGE as separation systems (NACE), obtaining very good resolution in a rather short analysis time [109–111].

Also 2-*O*-(2-aminoethyl-imino-propyl)-β-*O*-hydroxypropyl-β-CD (2-AIPHP-β-CD) [112] and 6-*O*-(2-hydroxy-3-trimethylammoniopropyl)-β-CD (6-HPTMA-β-CD) [113] were successfully used for the separation of several acidic compounds owing to their high solubility in water, high depth of the cavity and different stereoselective interaction pattern with analytes due to the presence of different functional groups on the rim of each compound.

The SID 6-mono-deoxy-6-mono-(2-hydroxy)-propylamino-β-cyclodextrin (IPA-β-CD) and 6-mono-deoxy-6-mono-(3-hydroxy)-propylamino-β-cyclodextrin (PA-β-CD) were used applying a reversed CE polarity (−30 kV), obtaining efficient separation of negatively charged compounds (profens) in a methanolic background electrolyte (NACE) [114]. The analytes detection was obtained by ESI-MS in the negative-ion mode. Using a negative separation voltage and a small hydrodynamic pressure on the capillary inlet, efficient chiral separation and selective detection of acidic drugs could be achieved, realizing a considerable improvement when compared with UV detection. MS detection was also used in a new chiral CE–MS approach where SID cationic CDs were used at very low concentrations for the resolution of fluorescein isothiocyanate amino acids (FITC-AAs) [115]. The usefulness of this chiral CE–MS method has been demonstrated through the study of different samples, in the field of the analysis of genetically modified organisms.

6-Mono-deoxy-6-mono-methylamino-β-CD (M-A-β-CD), 6-mono-deoxy-6-dimethylammino-β-CD (diM-A-β-CD) and 6-mono-deoxy-6-trimethylammino-β-CD (triM-A-β-CD) were used to separate 2,4-dinitrophenyl labelled amino acids (DNP-

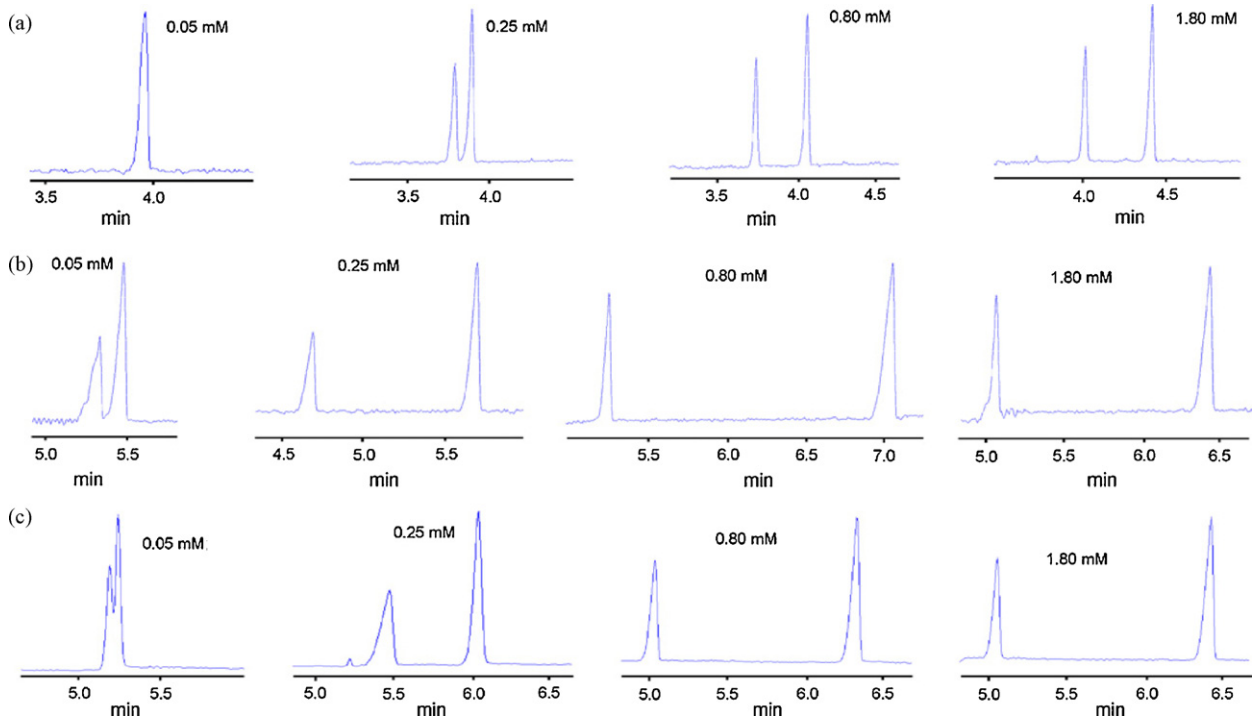

Fig. 6. Electropherograms (λ = 218 nm) of GCD3AM/Dns-leucine systems at four selected concentrations of GCD3AM: (a) pH 9.2 (20 mM borate buffer), (b) pH 6.8 (20 mM CH_3COONH_4), (c) pH 3.8 (20 mM acetic buffer). Capillary: 75 μm i.d., 50 cm/60 cm effective/total length, uncoated fused silica.
Reported with permission from Ref. [107].

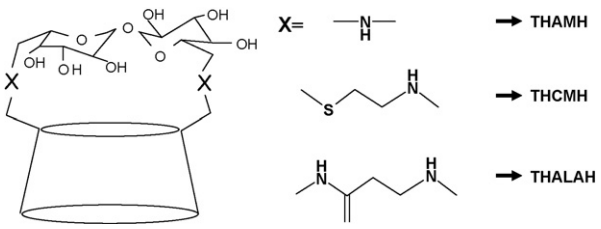

Fig. 7. Schematic structure of hemispherodextrins.

AAs) [116], either as single enantiomeric couple (triM-A-β-CD, M-A-β-CD), or as multicomponent mixtures of enantiomers (M-A-β-CD) and mixtures of positional isomers, indicating the importance of structural parameters (different degrees of methylation) of the studied chiral selectors in the separation mechanism. Analogously, monosubstituted, permanently dicationic β-CD, the mono-6-deoxy-6-*N*,*N*,*N*′,*N*′,*N*′-pentamethylethylenediammoniocyclomaltoheptaose (PEMEDA-BCD) [117] was used to study the charge effect on the enantiorecognition process.

Among monoamino-substituted derivatives, the permethylmonoamino-β-CD (PMMA-β-CD) seems to be an ideal chiral selector for pyrethroic acids in CD-EKC [118], readily dissolving these analytes in aqueous buffers even as neutral species due to its high methylation degree.

2.2.2. Capped derivatives

Though CDs are among the most used hosts in enantiorecognition, it can be hypothesised that the toroidal shape of their cavity does not provide the optimal separation of the guest molecule from the solvent. While the possibility of access of molecules into the cavity should obviously be preserved, CDs could work as well even by maintaining one-way access instead of two. Capped CDs [119] represent a class of CDs where, by bonding a chain to two different points of the cavity, a sort of bridge that interposes between the solvent and the cavity is obtained. In this context, our group used a new approach directed to reinforce the peculiar characteristics of the CDs, by including a saccharidic unit in the substituted chain [120–124]. This specific capping unit, not only physically better separates the cavity from the solvent, but, by extending the cavity dimensions with α,α′-D-trehalose, actually improves the CDs receptor characteristics. This class of hosts, whose schematic molecular structure is shown in Fig. 7, were named hemispherodextrins (HMs) due to the approximately hemispherical shape of the surface of these molecules when considering the saccharidic system of the cavity together with that of capping chain. Three hemispherodextrins, namely 6A,6D-(6,6′-diamino-α,α′-trehalose)-6A,6D-dideoxy-β-CD (THAMH), 6A,6D-dideoxy-6A,6D-[6,6′-dideoxy-6,6′-di(S-cysteamine)-α,α′-trehalose]-β-CD (THCMH) and 6A,6D-dideoxy-6A,6D-N-[6,6′-di-(β-alanylamido)-6,6′-dideoxy-α,α′-trehalose]-β-CD (THALAH) were used in CD-EKC, succeeding in the separation of phenoxy acids [120–121] as well as of arylpropionic acids [122]. In this last case, six different racemates of the profen family were separated either as single racemate, or as a mixture containing all the six enantiomeric pairs, showing that these receptors can also be used for achiral separation. The results obtained by adding a binary mixture of HMs to the background electrolyte show a complementary effect in chiral selectivity. Furthermore, THCMH and THALAH were used, at very low concentrations, to separate the enantiomeric pairs of 11 Dns-AAs [123,124], showing better resolution capabilities than the monosubstituted derivative 6-mono-deoxy-(2-aminoethylamino)-β-CD (CDen). The very large values of selectivity shown by hemispherodextrins, are probably due to the possibility of better isolating hydrophobic moieties of the substrate from the solvent by using a saccharidic "cap", giving rise to more selective interactions with substrates that differentiate very efficiently between the two members of an enantiomeric pair.

2.3. CLECE

A particular kind of EKC can be used in separations, characterised by the exploitation of the ligand exchange mechanism. By adding a metal complex in BGE, if the analyte has ligand properties towards that specific metal ion, it can substitute other ligands, including the solvent, in the coordination sphere of the metal ion. The different mobility of this complex with respect to the free analyte will influence the observed migration time. Further theoretical details can be found in reference [125].

Though the very first use of ligand exchange in separation science, in the context of liquid chromatography, dates back to the far 1961 [126], and in capillary electrophoresis to 1985 [127], it was not until 2003 that CLECE (chiral ligand exchange capillary electrophoresis) was obtained by adding, besides copper(II) ion, as chiral ligand in BGE, cyclodextrin derivatives [128]. By considering that parent CDs show poor coordination ability, the reason for this delay may be quite easily argued by the references already reported in this review. If we do an approximate statistics, we see that.

The use of SID is numerically very limited, at least in comparison to those using commercially available mixtures of derivatives.
The most of the separations by SID were obtained by sulphate derivatives, which, as known, have no appreciable coordinating ability.

It is neither surprising that the first used CD derivative was a base, due to the presence of two nitrogen atoms, one imidazolic and one aminic, thus undergoing two protonation equilibria. This selector summarises the characteristics necessary to be used in CLECE: being a chiral molecule and undergoing protonation equilibria, thus changing its electrical charge on the basis of the experimental conditions. The experimental conditions involved are not only the pH value of the BGE, but also the concentration of metal ion, which competes with the proton for the selector. Further, we would underline the importance, from one side, of the presence of nitrogen atoms in the selector in order to promote the complexation, and, from the other side, of using copper(II) as metal ion. Both of them show the highest flexibility, the nitrogen atom as donor atom, the copper(II) as metal ion, because they show good coordinating ability towards both hard and soft partners.

Other papers by our group followed, using CD derivatives in CLECE [129–131]. In this series of papers, by also exploiting our expertise in the study of equilibria in solution, it was possible to correlate the results of CLECE experiments to the species distribution of the analytes in BGE. Here, we will cite the results concerning the use of two strictly correlated β-CD 6-derivatives, namely the CDhm and CDmh (Fig. 1).

Both these selectors were previously synthesised and characterised [132–135]. On the basis of these results, and particularly of the observed stereoselectivity in the formation of their ternary copper(II) complexes with the tryptophan enantiomers evidenced by the values of their stability constants, CLECE experiments were carried out, obtaining for both selectors complete chiral separation. We would here underline the complete agreement between thermodynamic and electrophoretic data, showing how the EMO (electrophoretic mobility order) between the enantiomers, opposite for the two different selectors, is reflected in the corresponding magnitude order of their stability constants. In Fig. 8, it is reported the hypothesised schematic structure for the four complexes on the basis of an hypothesised "cis effect", accounting for the obtained experimental results. For the cited systems, the interpretation of the CLECE data appear quite straightforward.

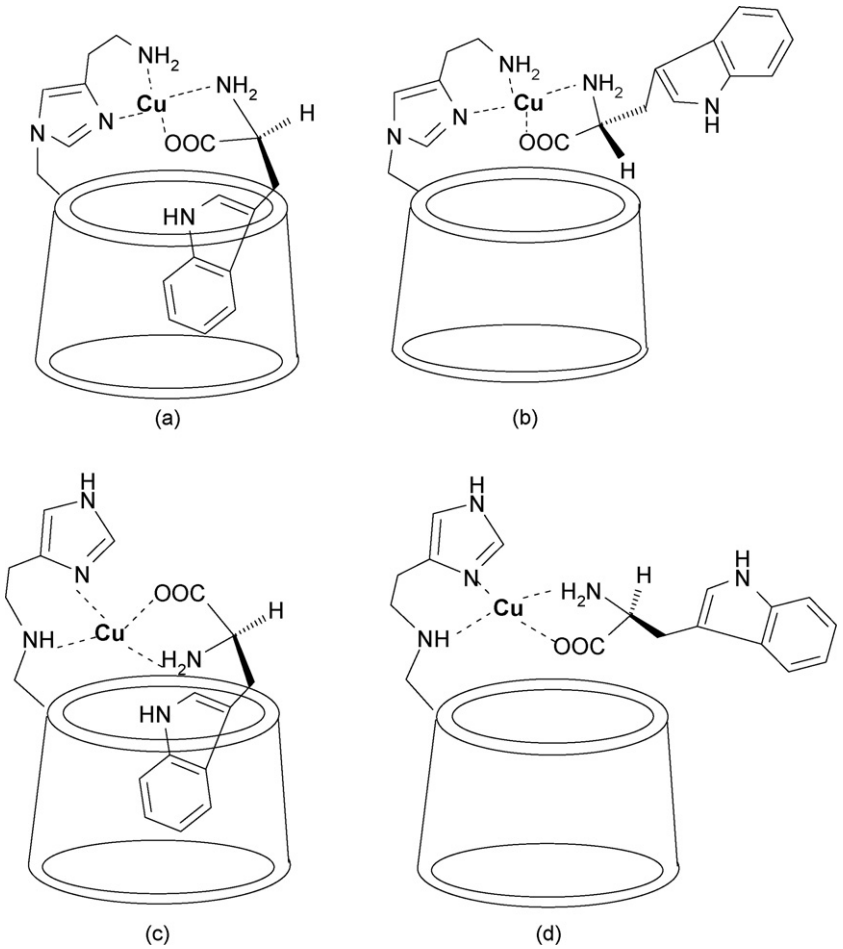

Fig. 8. Schematic structures of the complexes (charges omitted): (a) [Cu(CDmh)(L-Trp)]; (b) [Cu(CDmh) (D-Trp)]; (c) [Cu(CDhm)(D-Trp)]; (d) [Cu(CDhm)(L-Trp)]. Stability constants of these species are respectively: 18.99 (a), 18.14 (b), 16.47 (c), and 16.12 (d).
Reported with permission from Ref. [131].

More complex cases are those reported in references [129] and [130], where at the pH value of the BGE, more species are present in significant percentages. For example, the species distribution for the system Cu(II)/CD3NH2/phenylalanine [129], is shown in Fig. 9. In this case, the substitution occurs at a secondary position of the glucopyranosinic ring. As known, the acidity of the secondary hydroxyls is higher and, differently from the analogous primary groups, in the presence of a suitable metal ion and of a suitable steric disposition, they can be deprotonated even at quite low pH value. Thus, the strong donor properties of the amino nitrogen give rise to the bonding of the copper(II) ion. Favourable steric conditions occur that permit to the deprotonated 2-hydroxyl to bond the ion as confirmed by the stability constants obtained by pH-metric potentiometry. Thus, in the ternary systems with each enantiomer of phenylalanine, both the unprotonated $[Cu(CD3NH2)(Phe)]^{+}$ and the deprotonated $[Cu(CD3NH2)(Phe)H_{-1}]$ ternary species are simultaneously present. It is important once more to carefully consider the electric charge of the complexes. Since the analyte is neutral at the BGE pH, only the unprotonated species, being cationic, can differentiate the migration times between the phenylalanine enantiomers. As shown in the distribution diagram, the L-enantiomer unprotonated complex is formed in higher percentage, due to its higher stability constant, and correspondingly, the L-enantiomer has a shorter migration time as observed in the electropherograms.

The electropherograms reported in Fig. 10 for the system Cu(II)/AB3NH2/tyrosine give evidence of the excellent separations obtained.

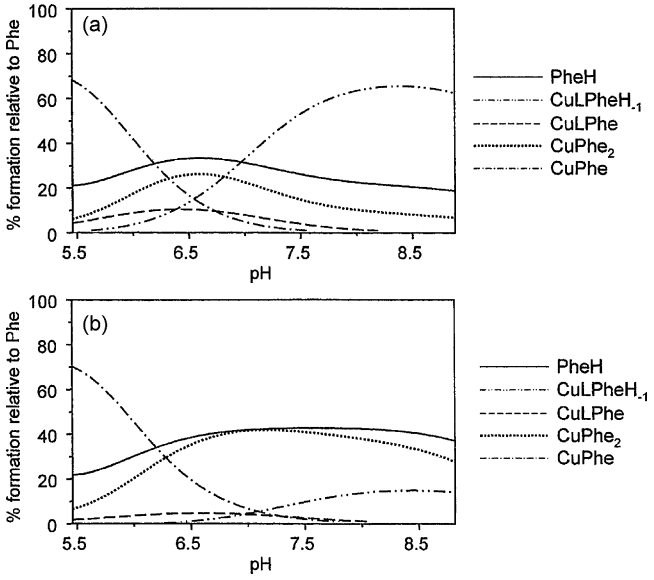

Fig. 9. Distribution diagram for the Cu^{2+}–CD3NH2–AaO^{-} system: $[Cu^{2+}] = 7.5$ mM, [CD3NH2] (L) = 9.0 mM, [Phe] = 0.1 mM: (a) L-phenylalanine; (b) D-phenylalanine.
Reported with permission from Ref. [129].

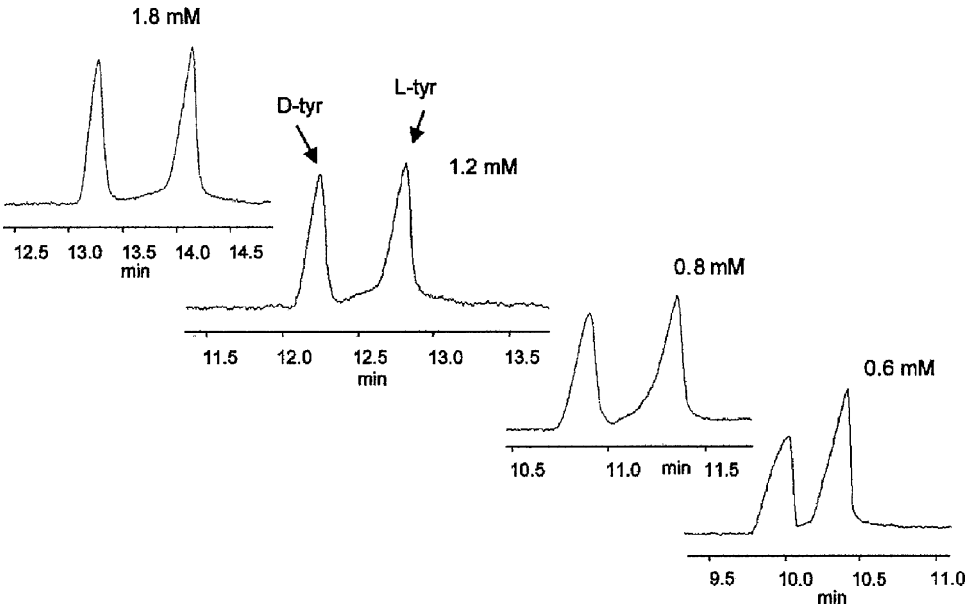

Fig. 10. Electropherograms of tyrosine racemate in the presence of AB3NH2 (0.6–1.8 mM) and of $CuSO_4$ ([Cu^{2+}]–AB3NH2 1: 1.2 ratio) in 20 mM CH_3COONH_4 (pH 6.8). The system operated at a constant voltage of 12 kV. Capillary: 50 μm i.d., 37.5 cm/50 cm effective/total length, uncoated fused silica.
Reported with permission from Ref. [130].

The CLECE results obtained by CD derivatives are summarised in Table 3, mostly derived from paper [131]. We would underline the range of very low concentrations used in this series of studies, and how in some cases the highest concentration used is not the best, as resulting from the selectivity values reported. If coupled to a low absorptivity value of the complex selector–metal ion in the BGE, it results, at a low concentration, in a low absorbance which does not disturb the baseline in the electropherograms. Further, considering

Table 3
Selectivity (S) and resolution (R) values of CLECE separations of Phe, Trp and Tyr enantiomers with some selected cationic SID of β-cyclodextrin derivatives at different concentrations.

	C (mM)	Phe		Trp		Tyr	
		S	R	S	R	S	R
CD3NH2	1.8	0.015	1.45			0.015	1.33
	1.2	0.023	1.71			0.009	0.47
	0.8	0.012	0.54			0.007	0.39
	0.6	0.005	[a]				
CDen	1.8			0.006	[a]		
	1.2	0.01	[a]	0.015	[a]	0.042	1.26
CD3en	0.8			0.031	0.63		
	0.6			0.02	[a]		
βCDhm	1.8			0.02	1.15	0.014	1.07
	1.2			0.019	1.27	0.007	[a]
	0.8					0.005	[a]
Cdampy	1.8	0.009	[a]			0.031	1.18
	1.2					0.023	0.92
	0.8	0.015	[a]			0.012	0.91
	0.6					0.024	0.88
ABNH2	1.8			0.01	0.65	0.015	1.09
	1.2					0.014	0.8
AB3NH2	1.8	0.027	0.86	0.031	1.31	0.063	3.22
	1.2	0.017	0.45	0.029	0.95	0.046	2.42
	0.8	0.04	0.41			0.04	2.57
	0.6	0.009	[a]			0.039	1.7
βCDmh[b]	1.8						
	1.2			0.017	0.8		
	0.8			0.014	0.53		
	0.6			0.018	0.95		
	0.4			0.029	1.76		
	0.25			0.036	1.52		
	0.15			0.044	1.07		
	0.1			0.037	0.71		
	0.07			0.029	0.62		

[a] Low value.
[b] CLECE experiments were carried out only for Trp.

the cationic charge of the selector at that pH, a low concentration minimises its interaction with the capillary wall, avoiding effects of fronting and tailing.

Other papers were reported from another group, but using fairly higher concentrations of the selector [136,137], included, as cationic selectors, in Table 2.

3. Concluding remarks

Writing this review, we fully realised the excellent situation of scientific literature in the field of the chiral separations in CE, both as quality of the separations, and of the increasing number of investigations carried out. Further, it appears clear that the role of cyclodextrins in this field remains unsurpassed, by their easy availability and of the flexibility in their use.

The different characteristics inherent to permanently ionic CDs, from one side, and ionisable CDs from the other side, have been already underlined in the introduction.

As concerns the specific subject of this review, we tried to summarise the advantages and also the disadvantages of the use of SID derivatives, with respect to the mixtures of derivatives commercially available. As an overall balance, we would say that the unavoidable and undoubted synthetic efforts necessary to obtain them is widely rewarded by a detailed knowledge, not otherwise obtainable, of what is going on during the electrophoretic run. This, in turn, permits what we call a rational approach to CE, by predicting the influence of at least some of the experimental parameters on the results of the separation carried out. At the same time, this encourages to carry out, by using supplementary techniques, the characterisation of the molecular chiral recognition process between the selector and the analytes, rather than to simply obtain the wanted separation. We would strongly recommend the use of spectrophotometric and potentiometric techniques, with the specific aim to thermodynamically characterise the investigated systems, and thus to obtain the detailed knowledge of the equilibria occurring in solution during the electrophoretic run.

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